THE SYNTHESIS AND CHARACTERISATION OF LONG-CHAIN FATTY ACIDS

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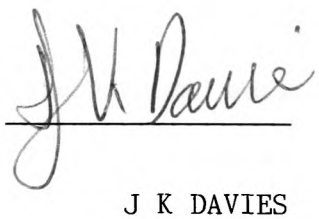
DECLARATION

This thesis has not been nor is currently submitted for the award of any other degree or similar qualification.



J K DAVIES

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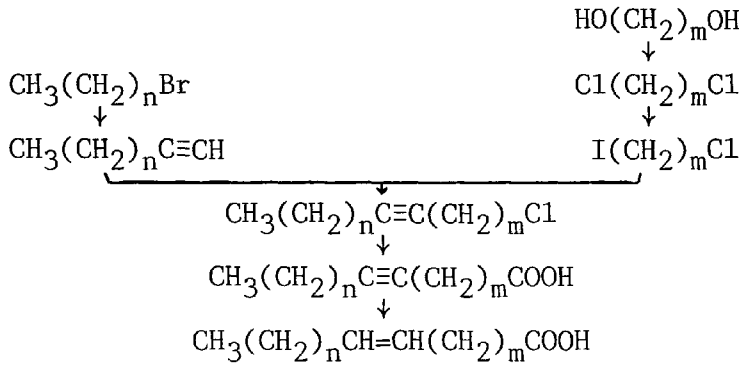
ABSTRACT

THE SYNTHESIS AND CHARACTERISATION OF LONG-CHAIN FATTY ACIDS. J K DAVIES

The commercial hydrogenation of oils and fats results in the formation of, among other components, geometrical and positional isomers of monounsaturated fatty acids. The view that there may be hazards associated with such oils have occasionally been expressed. Prior to an investigation of the levels of such acids in human tissue and UK dietary fats, the synthesis of a series of these acids, as standards, and their characterisation, was necessary.

For the synthetic programme, the general scheme:-



provided a convenient route for the synthesis of geometrical and positional isomers from common precursors. When condensation of the 1-alkyne and α-chloro-ω-iodoalkane was performed via sodamide in liquid ammonia, the scheme was limited but was extended somewhat when performed via methyllithium in dioxan. Generally, yields decreased with the increasing chain length, and migration of the unsaturated bond to the extremities of the resulting molecule.

Chromatographic separation, both capillary column GLC and reverse-phase HPLC, was readily achieved on the basis of chain length and configuration of the double bond. Furthermore, the partial separation of positional isomers was achieved.

NMR spectroscopy unambiguously determined the configuration and position of unsaturation in virtually every fatty acid. Assignment is based on the fact that functional groups within an acid alter the chemical shift of neighbouring carbons in a characteristic manner.

No one of these techniques alone is applicable to the determination of positional isomerism in a complex lipid mixture and must be used in combination. Whereas NMR is undoubtedly invaluable in the quantification of positional isomers on an individual basis, or in profiling simple mixtures, it is not as applicable to the direct analysis of complex lipid samples. Emphasis on the continuing development of capillary column GLC holds the most promise for the direct quantification of positional isomerism.

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LIST OF ABBREVIATIONS

AOCS	American Oil Chemists Society
BHT	Butylated hydroxytoluene
BHQ	Butyl hydroquinone
$CDCl_3$	Deuterated Chloroform
CS_2	Carbon Disulphide
CW	Continuous Wave
D_2O	Deuterium Oxide
EFA	Essential Fatty Acid(s)
FAME	Fatty Acid Methyl Ester(s)
FID	Flame Ionisation Detector
FT	Fourier Transform
GLC	Gas Liquid Chromatography
H	Higher acids - the sum of acids 20:0, 20:1, 20:2, 20:3, 22:0 and 22:1
HF	Hydrogenated Fat
HMO	Hydrogenated Marine Oil
HPLC	High Performance Liquid Chromatography
HVO	Hydrogenated Vegetable Oil
Hz	Hertz
IHD	Ischaemic Heart Disease
IR	Infrared
L	Lower acids - the sum of acids 14:1, 15:0, 15:0br, 15:1, 16:0br, 17:0 and 17:1
M	Mega
MAFF	Ministry of Agriculture, Fisheries and Food
MS	Mass Spectrometry
NFS	National Food Survey

NMR	Nuclear Magnetic Resonance
ppm	parts per million
PUFA	Polyunsaturated Fatty Acid(s)
RAF	Ruminant Animal Fat
T	Total trans Unsaturated Acids
T_H	Higher trans acids - the sum of trans acids of chain length C_{20} and C_{22}
T_L	Lower trans acids - the sum of trans acids 16:1 and 18:1
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsialane
UK	United Kingdom
US	United States
UV	Ultraviolet

PART ONE

INTRODUCTION

SECTION ONE

GENERAL INRODUCTION

1 Lipid Classification

The main sources of dietary fatty acids are mainly triacylglycerols, with smaller amounts of mono- and diacylglycerols, phospholipids and cholesterol esters. These compounds belong to a class collectively known as lipids. The term lipid has traditionally been used to describe a wide variety of natural products,[1] although today, it is frequently restricted to fatty acids and their naturally-occurring derivatives and to compounds biosynthetically related to fatty acids. It is in this context that the term is used here.

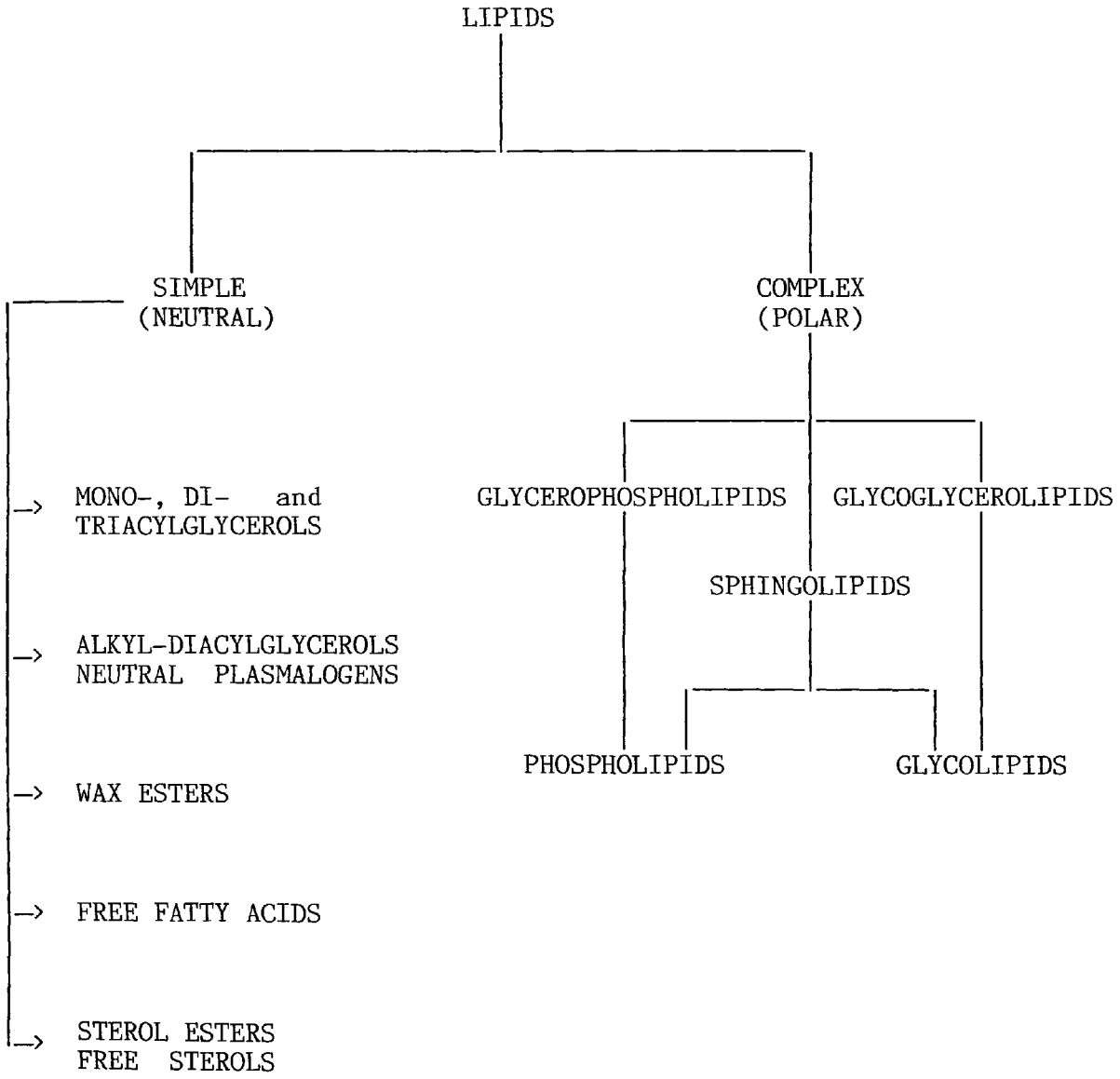
Lipids may be subdivided into two broad classes - "simple" and "complex". The terms "neutral" and "polar" respectively are used more frequently to define these classes but are less precise and may occasionally be ambiguous. Within these two broad classes, lipids may be further subdivided into various other classes (Figure 1).

1.1 Simple Lipids

Generally, simple lipids contain only fatty acid and alcohol components. The alcohol is usually glycerol but it may also be a long-chain alcohol (wax esters) or a sterol (e.g. cholesterol esters). Esters of C_2, C_3 and C_4 diols are also known but are rarely found in greater than trace amounts.[2] Fatty acids and sterols such as cholesterol (in animals) and ergosterol (in plants) are also classed as simple lipids.

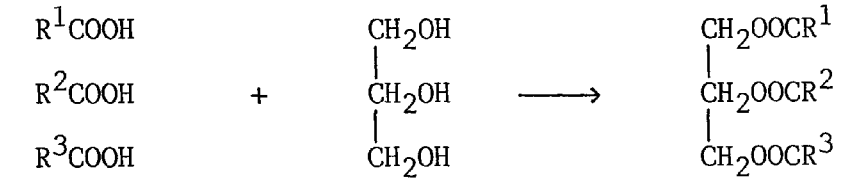
Triacylglycerols belong to this class and are by far the most abundant single lipid class. They consist of three fatty acid moieties linked to the trihydric alcohol glycerol (Figure 2). Virtually all the commercially important fats and oils of animal or plant origin and most animal depot fats consist entirely of this lipid. The composition and

FIGURE 1
Lipid Classification



properties of triacylglycerols have been comprehensively reviewed.[3]

FIGURE 2
Glyceride Structure



Mono- and diacylglycerols which contain one or two moles per mole of glycerol respectively, are often collectively termed as partial glycerides. They are rarely present in greater than trace amounts in fresh animal and plant tissues, but some have particular importance biosynthetically as precursors of triacylglycerols and complex lipids.

Alkyl-diacylglycerols, as the name suggests, are lipid components in which a long chain alkyl group is bonded by an ether linkage to glycerol; the other two positions being esterified with conventional fatty acids. They occur in small amounts in animal tissues and are occasionally found in large quantities in the lipids of marine animals. Related compounds are neutral plasmalogens where glycerol is linked to an alkyl group via a vinyl ether bond. Such lipids have, however, been detected in small amounts only in certain animal tissues.

Other members of this class are the wax esters which commonly consist of fatty acids esterified to long chain alcohols. Wax esters are found in animal (e.g. skin lipids) and insect (e.g. beeswax) secretions. They also occur as protective coatings on plant leaves and fruits, in the lipids of algae, fungi and bacteria. Additionally, they are sometimes

major components in the depot fat of marine animals.

1.2 Complex Lipids

Complex lipids may be subdivided into three main classes: glycerophospholipids, glycoglycerolipids and sphingolipids.

Glycerophospholipids are composed of glycerol, fatty acids, inorganic phosphate and an organic base or polyhydroxy compound. They are found in all plant and animal tissues and in microorganisms. The most common glycerophospholipid is phosphatidylcholine, commonly termed "lecithin". It is more often than not the most abundant glycerophospholipid in animal tissues and is often a major lipid of plant tissues and of microorganisms.

Glycoglycerolipids are composed of glycerol, fatty acids and carbohydrates and are almost entirely plant and bacterial lipids although trace amounts have been found in the brain tissues of some mammalian species.

Sphingolipids contain a long-chain base, fatty acids and inorganic phosphate, carbohydrates or other complex organic compounds. They are mostly important constituents of animal tissues, although they have also been found in plants and some microorganisms.

Two terms often associated with complex lipids are glycolipids and phospholipids. The term glycolipid is used to describe any compound containing one or more monosaccharide residue linked by a glycosyl linkage to a lipid part. It therefore encompasses glycoglycerolipids and certain of the sphingolipids. The term phospholipid denotes any lipid containing phosphoric acid as a mono- or diester and so includes

glycerophospholipids and the sphingolipid, sphingomyelin.

Lipid classification is comprehensively covered in a number of literature sources.[4,5,6]

2 Diverse Roles of Dietary Lipids

Body lipids can be classified broadly as "structural" or "storage". Structural lipids are those that form an integral part of the biological membranes found in all cells, tissues and organs. In man they are mainly the phospholipids, glycolipids and cholesterol. Storage lipids are those that provide a long term reservoir of energy in the adipose tissue and are almost exclusively triacylglycerols.

The roles of dietary lipids however are more than that of just structure and storage. A completely fat free diet would be extremely unappetising and probably, in view of the need for EFA in the diet, very harmful.[7] Some lipids are essential components of certain enzyme systems. Lipids also affect the texture of food. As a result of their emulsifying properties, complex lipids such as the lecithins, and simple lipids such as the mono- and diacylglycerols, not only help in the emulsification of food in the gut, but are used in the food industry to aid in the emulsification and stabilisation of foods prior to consumption. Lipids also act as carriers for fat-soluble vitamins which are more efficiently absorbed if there is sufficient fat in the diet to carry them. Finally, many of the aromas that stimulate an appetite for food are volatile breakdown products of lipids.

3 Fatty Acid Structure and Nomenclature

3.1 Structure

The basic unit of the visible fats of animal tissue or the seed oil of plants is the triacylglycerol. Oils are liquid and fats are solid at room temperature. Whereas the glycerol component is a constant feature, several related families of fatty acids exist, the nature of which can have a profound effect on the physical and chemical nature of, not only the triacylglycerol itself, but also the fat or oil with which it is associated. Triacylglycerols may be termed homogeneous or heterogeneous, depending on whether the three acid moieties are identical or different.

Structurally, fatty acids are hydrocarbon chains terminating in a carboxyl group. The systematic name is derived from the parent hydrocarbon by the substitution of -oic for the final -e. The first three members of the series exhibit no fatty characteristics but simply fit the structural pattern of the series, and even the fourth member only qualifies since it is found combined in butter fat, yet does not itself show typical properties such as insolubility in water. With the sixth member of this series, hexanoic acid, this fatty property is exhibited and the acid is found in the oils of various species of palm.

Fatty acids with an even number of carbon atoms predominate in natural fats by virtue of the metabolic process through which they are formed, although odd-numbered and branched-chain acids have also been isolated. Whereas fatty acids of animal origin are comparatively simple in structure, plant fatty acids may be more complex and contain a variety of other functional groups.

Chemical and physical properties of fatty acids are largely determined by their chain lengths and degree of unsaturation. The most widespread and important fatty acids are the C_{16} and C_{18} acids. However, in animal fats - C_{14} acids, certain plants - C_{6-12} acids and, in the oils of fish and other marine animals, C_{20} or longer chain acids are not uncommon.

Fatty acids may be fully saturated or contain between one and six double bonds. The incorporation of double bonds into the molecule gives rise to geometrical (*cis* and *trans*) and positional (the distribution along the alkyl chain) isomers which affect the chemical properties, both of the acid itself and the glyceride with which it is associated.

3.2 Nomenclature

To simplify presentation and discussion of fatty acid compositions, fatty acid nomenclatures exist. In the simplest form, fatty acids are designated solely by the number of carbon atoms they possess e.g. C_{18}. A full description of any acid, however, must specify chain length, degree of unsaturation, position and configuration of unsaturation. Consequently, saturated, monounsaturated and polyunsaturated fatty acids such as stearic, oleic and linoleic acids may be designated 18:0, 18:1Δ9c and 18:2Δ9c,12c respectively, indicating the position and configuration of the double bond where the carboxyl carbon is C-1. Alternatively, and more recently, the stereochemistry of the double bond has been designated in terms of E and Z forms.[8] This system of nomenclature uses a system of sequence rules to assign priorities to the substituent groups on the double bond carbons. However, the *cis/trans* nomenclature is used in this study as it is unambiguous and quite acceptable for all disubstituted alkenes. In the nomenclature used here, the position of unsaturation is denoted in parentheses followed by either a, c or t

depending on whether unsaturation is acetylenic, *cis* or *trans* e.g. *trans*-8-hexadecenoic acid is designated 16:1(8)t. Alternatively, the position of the double bond can be denoted in the form (n-x) where n = acid chain length and x = number of carbon atoms from the last double bond to the terminal methyl group. For example, oleic acid is 18:1(n-9) and linoleic acid is 18:2(n-6) (assuming the double bonds are methylene-interrupted). This nomenclature is only used in fatty acids containing *cis* double bonds. IUPAC-IUB Commissions[9,10] have reluctantly agreed to this form of nomenclature because of its convenience to biochemists with interests in fatty acid metabolism but it is not used here except for the description of polyunsaturated acids in Table 3 (it is more convenient to write 22:6(n-3) than 22:6Δ4c,7c,10c,13c,16c,19c).

3.3 Saturated Fatty Acids

Saturated fatty acids (Figure 3a) are comparatively inert chemically and lipids containing only these acids can be subjected to more vigorous conditions than unsaturated fatty acids. Table 1 contains a summary of the commonest saturated fatty acids together with their trivial names, shorthand designations and some common sources of origin.

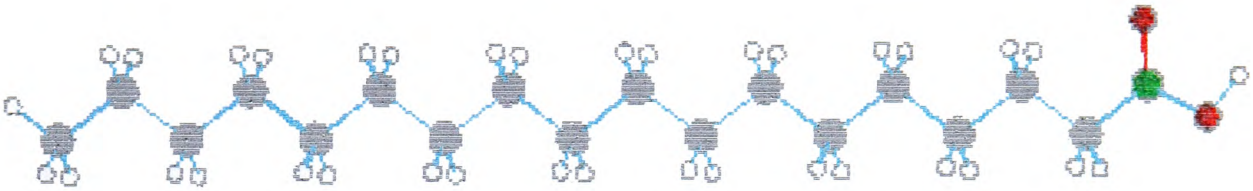
3.4 Monounsaturated Fatty Acids

Monoenoic acids and lipids containing such acids are more susceptible to chemical attack, particularly by oxidising agents, than the corresponding saturated compounds. They are fairly resistant to autoxidation, but will succumb to this under very vigorous conditions. The double bond is normally in the *cis* configuration (Figure 3b) in naturally occurring acids, though acids with the *trans* configuration (Figure 3c) are also known but occur comparatively rarely. Monoenoic acids are mostly all low melting point compounds, the *trans*-isomers having slightly higher

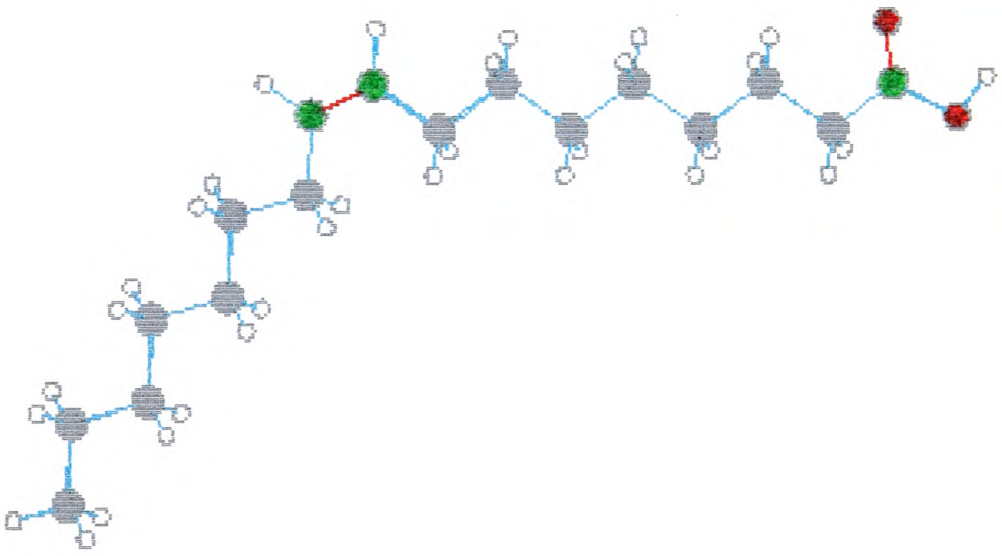
FIGURE 3 Fatty Acid Structures

Black Atoms	= sp^3 Carbons	White Atoms	= Hydrogen
Green Atoms	= sp^2 Carbons	Red Atoms	= Oxygen
Light Blue Bonds	= Single Bonds	Red Bonds	= Double Bonds

a) Octadecanoic (Stearic) Acid

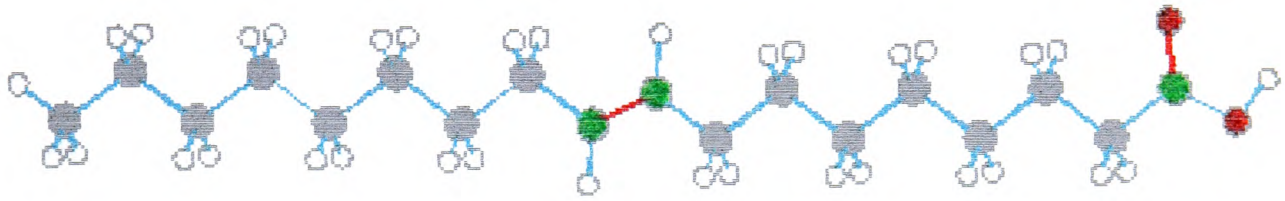


b) *cis*-9-Octadecenoic (Oleic) Acid



Chem-X, developed and distributed by Chemical Design Ltd,
Oxford, England

c) *trans*-9-Octadecenoic (Elaidic) Acid



d) *cis,cis*-9,12-Octadecadienoic (Linoleic) Acid

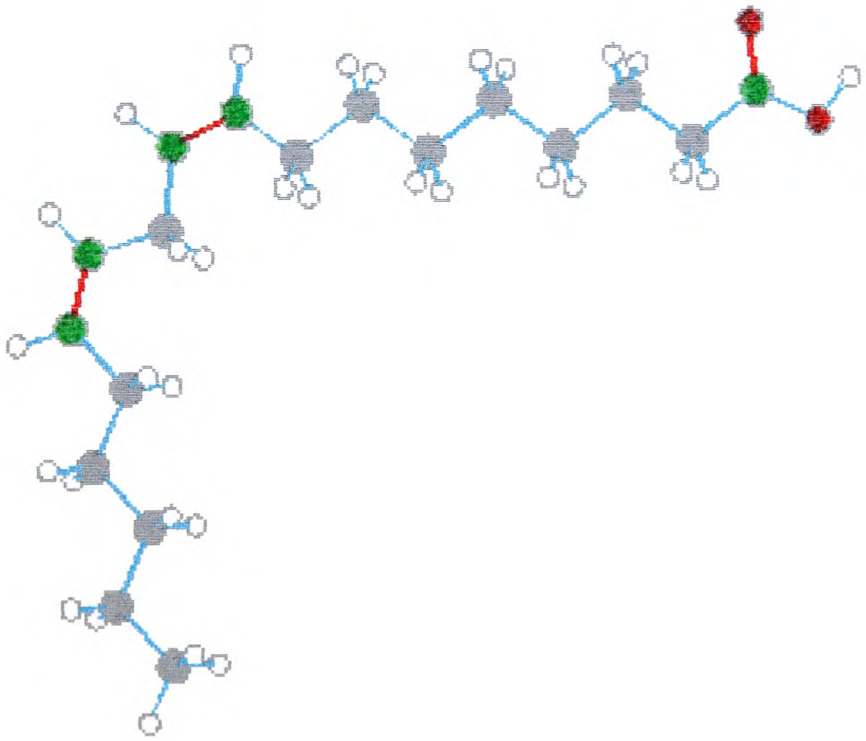


TABLE 1

Some Saturated Fatty Acids of General Formula $CH_3(CH_2)_nCOOH$

Systematic Name	Trivial Name	Shorthand Designation	Sources and Remarks
Butanoic	Butyric	4:0	Milk and butter fats.
Hexanoic	Caproic	6:0	Milk fats and some palm oils.
Octanoic	Caprylic	8:0	
Decanoic	Capric	10:0	Milk fats and various seed oils.
Dodecanoic	Lauric	12:0	
Tetradecanoic	Myristic	14:0	Minor component of most animal lipids and major component in some seed oils.
Hexadecanoic	Palmitic	16:0	Commonest saturated fatty acid. Found in virtually all animal and plant fats and oils.
Heptadecanoic	Margaric	17:0	Bacterial lipids.
Octadecanoic	Stearic	18:0	Also very common. Sometimes more abundant than 16:0 especially in complex lipids.
Eicosanoic	Arachidic	20:0	Often major components of waxes. Occur in most animal lipids but can occur in larger quantities in certain marine animals and bacterial lipids.
Docosanoic	Behenic	22:0	
Tetracosanoic	Lignoceric	24:0	

melting points than their *cis* analogues. In addition to the configuration of the double bond, its position may vary from acid to acid depending on the source. Additionally, animal lipids frequently contain monoenoic fatty acids that are biosynthetically related.[11] That is, several components may arise by chain elongation or chain shortening of a common precursor. The more common naturally occurring monoenoic fatty acids together with their trivial names, shorthand designations and common sources of origin are summarised in Table 2.

3.5 Polyunsaturated Fatty Acids

Fatty acids containing two or more double bonds are classed as polyunsaturated fatty acids (PUFA) (Figure 3d). As with monoenoic fatty acids, these acids may be subdivided into several simple families according to their biosynthetic derivation from single specific fatty acid precursors.[11] The double bonds of these acids are normally of the *cis*-configuration which are generally interrupted by a single methylene group (methylene-interrupted or non-conjugated). These acids all have very low melting points and, the greater their degree of unsaturation, the greater their susceptibility to autoxidation. The more important of these acids together with their trivial names and shorthand designations are summarised in Table 3.

3.6 Other Fatty Acids

Branched-chain fatty acids are common constituents of bacterial lipids,[12] but can enter the food chain and appear in animal tissues. Such acids are characteristic of RAF and are formed by biohydrogenation by bacteria present in the ruminant stomach. Those found most frequently have a single methyl group on the penultimate (*iso*) or antepenultimate (*anteiso*) carbon atoms. As they are generally fully saturated compounds,

TABLE 2

Some Monoenoic Fatty Acids of General Formula $CH_3(CH_2)_nCH{=}CH(CH_2)_mCOOH$

Systematic Name	Trivial Name	Shorthand Designation	Sources and Remarks
cis-9-Dodecenoic	Lauroleic	12:1Δ9c	Milk fats.
cis-9-Tetradecenoic	Myristoleic	14:1Δ9c	Traces in ruminant animal fat (RAF).
trans-3-Hexadecenoic	-	16:1Δ3t	Seed oils.
cis-9-Hexadecenoic	Palmitoleic	16:1Δ9c	Present in most animal fats and marine oils.
cis-6-Octadecenoic	Petroselinic	18:1Δ6c	Seed oils of the Umbelliferae.
cis-9-Octadecenoic	Oleic	18:1Δ9c	Probably the most abundant fatty acid of all. Found in virtually all lipids of animal and plant origin.
trans-9-Octadecenoic	Elaidic	18:1Δ9t	Rarely found in natural lipids.
cis-11-Octadecenoic	*cis*-Vaccenic	18:1Δ11c	Major unsaturated acid in many bacterial species.
trans-11-Octadecenoic	*trans*-Vaccenic	18:1Δ11t	Present in RAF due to biohydrogenation of PUFA by microorganisms in the rumen.
cis-9-Eicosenoic	Gadoleic	20:1Δ9c	Minor components of most animal lipids. Found in appreciable quantities in certain seed oils and in fish oils.
cis-11-Eicosenoic	Gondolic	20:1Δ11c	
cis-13-Docosenoic	Erucic	22:1Δ13c	
cis-15-Tetracosenoic	Nervonic	24:1Δ15c	

TABLE 3

Some Polyunsaturated Fatty Acids of General Formula $CH_3(CH_2)_n(CH{=}CHCH_2)_x(CH_2)_mCOOH$ (the double bond configuration in each case is *cis*)

Systematic Name	Trivial Name	Shorthand Designation	Sources and Remarks
9,12-Octadecadienoic	Linoleic	18:2(n-6)	Commonest and simplest acid of this type. Found in most plant and animal tissues. It is an EFA in animal diets as it cannot be synthesised by the animal yet is required for growth, reproduction and healthy development. In animal tissues it is the precursor of a family of other fatty acids which are produced from it by desaturation and chain elongation. All have the (n-6) terminal structure.
6,9,12-Octadecatrienoic	γ-Linolenic	18:3(n-6)	Important intermediate in the biosynthesis of arachidonic acid. Occurs in minor amounts in animal tissues but appreciable amounts in some seed oils.
8,11,14-Eicosatrienoic	Homo-γ-Linolenic	20:3(n-6)	-
5,8,11,14-Eicosatetraenoic	Arachidonic	20:4(n-6)	Most important of the 18:2(n-6) metabolites. Major component of animal tissue complex lipids but rarely found in plants. It is one of the principal precursors of the prostaglandins. It is an example of an essential metabolite (as are all fatty acids derived from EFA), but is not, as linoleic is, an essential nutrient.

(continued)

TABLE 3 Continued

Systematic Name	Trivial Name	Shorthand Designation	Sources and Remarks
4,7,10,13,16-Docosapentaenoic	-	20:5(n-6)	-
9,12,15-Octadecatrienoic	α-Linolenic	18:3(n-3)	Major component of plant photosynthetic tissues but rarely found in animal tissues. Important as the precursor of the (n-3) family of fatty acids which are essential fatty acids in fish.
5,8,11,14,17-Eicosapentaenoic	-	20:5(n-3)	Found in many animal tissues as major components of the complex lipids and they are also found in large amounts in fish oils.
4,7,10,13,16,19-Docosahexaenoic	-	22:6(n-3)	
5,8,11-Eicosatrienoic	-	20:3(n-9)	Normally a minor component of animal lipids but assumes importance in complex lipids deficient in EFA. Its biosynthetic precursor is oleic acid.

branched-chain acids are comparatively resistant to chemical degradation.

In addition to the more common fatty acids, plant lipids may contain a large variety of unusual fatty acids not found in the animal kingdom. The structures of these have been comprehensively reviewed.[13] Some of the functional groups found in these acids include acetylenic bonds, conjugated acetylenic and ethylenic bonds, allenic groups, cyclopentene and furan rings, double bonds of both *cis*- and *trans*-configurations and double bonds separated by more than one methylene group. Two or more of these functional groups may on occasion be found in a single fatty acid. Certain of these functional groups may be destroyed by some chemical techniques used widely in the analysis of animal lipids. Although fatty acids with these uncommon functional groups are most often found in plant lipids, they may on occasion be detected in animal tissues after injestion.

4 Consumption of Dietary Lipids With Particular Reference to Dietary Trends in the United Kingdom

4.1 Consumption of Fats in the United Kingdom

Our edible oils and fats are obtained from oilseeds, animals, oil-bearing trees and marine animals and are principally consumed as "visible" and "invisible" fats. Visible fats are derived from the depot tissue of animals or the seed oils of plants and are present in large amounts (almost exclusively as triacylglycerols) in butter and margarines, cooking fats and oils and the fat on meat. Invisible fats derive from such sources as milk, nuts, lean meat and other animal and vegetable foods. They constitute such components as the membrane phospholipids of beef or liver and the chloroplast phospholipids and

glycolipids of vegetables such as cabbage or lettuce.

There are considerable differences between countries in the types of oils and fats used. These arise from consumer preferences, the availability of indigenous oilseeds and the extent of livestock production. The predominant features are summarised in Table 4.

In contrast to other regions which rely heavily on one type of fat, Northern Europe, as a result of close ties with former colonies, tends to use a much broader spectrum of oil types.

The UK is no exception to the North European pattern and the oils and fats market is based on a wide range of oils and fats with no single oil representing more than about 20% of consumption. Total fat intake in the UK is calculated to be 2.6 million tonnes per year. About 40% of this can be identified as being from production and imports of refined edible oils used by the food industry in manufactured products. The bulk of the fats, 60%, is from other sources but primarily from meat and dairy products.[14]

Although the fatty acid composition from meat and dairy products remains on the whole fairly consistent,[15] the fatty acid composition of manufactured foods may vary considerably depending on the blend of fats used in their manufacture. Factors that may influence that blend include availability, the pattern of oilseeds crushed, indigenous oilseed production, price and consumer demand for the food. The range of fats typically used in food products are shown in Table 5.

As a rule, most manufacturers depend on blends of oils and fats to provide the least cost blend that will give the required performance in

Table 4
World Patterns of Oil Demand

Region	Dominant Oil or Fat
North and South America	Soybean
South-east Asia	Palm
Canada	Rapeseed
Australasia	Animal fats
Southern Europe	Olive and Groundnut
Northern Europe and UK	Fish, soybean, palm, rape, etc

Table 5
Types of Refined Fats Used in Manufactured Foods

Food	Main Oils Used
Margarine	Marine, palm, soybean, rape, sunflower, beef.
Cooking fats	Marine, palm, soybean, rape.
Cooking oils	Soybean, rape, sunflower, maize.
Biscuits	Marine, palm, rape, soybean.
Industrial frying	Soybean, palm, palm oleate, rape, cottonseed.
Chocolate	Fractionated fats, palm kernel, coconut.
Salad cream	Maize, soybean, sunflower.
Ice cream	Palm kernel, palm.

the food product and thereby keep down the cost to the consumer. An example of this is margarine and cooking fat production in the UK. Several types of oils are used for the production of margarine and cooking fats of which the main are HMO and HVO. Popular brands of margarines and cooking fats purchased during the periods 1976-1977 and 1979-1980 and analysed for fatty acid composition from which the blend of the source fats present could be characterised, were found to vary not only from brand to brand but also for a given brand over a period of time.[15]

On an individual basis of consumption per person, information of dietary trends in the UK this century is poor. Since the Second World War, there has been a regular system of monitoring the population and hence the pattern of change on a national basis in the form of the MAFF National Food Survey Committee .[14] Prior to that, however, general population data are based on very crude estimates which are often based on economic indices of food moving into consumption. Changes in the pattern of food distribution through the rise of major marketing organisations, food manufacturers and supermarkets in the last 20-30 years, makes pre- and post-war statistics on food consumption incomparable.

Before 1950, such data as are available suggest that fat intake increased from 98g in 1903 (all values quoted are expressed in terms of grams/person/day) to 133g in 1950, and that the percentage energy derived from fat increased from 32% to 39% between 1903 and 1938. This then remained comparatively constant at about 36-38% upto 1950.[16]

There are today two sets of data commonly cited as evidence for the changing fat content of the UK diet: (i) the Food Consumption Level

Estimates and (ii) the National Food Survey (NFS). Both are issued annually by MAFF. The Consumption Level Estimates are based on the total national production of food in the UK together with allowances made for imports and exports, whereas the NFS is derived from food entering the households in the UK. The NFS estimate is not, however, complete because it does not include alcoholic drinks, sweets or chocolate and does not make full allowance for food eaten outside the home (considered to amount to 15% of total energy). Table 6 summarises data from both sources on the fat content of the UK diet for the period 1952-1982.[14]

Both sources in Table 6 tend to suggest that after an initial increase following de-rationing, that since 1969, there has been a decline in both total energy and fat consumption. For the same period, the amount of energy derived from fat, after an initial increase, has remained constant. This condition is, however, difficult to substantiate because of the many differences between studies including differences in age, sex, social-class and region, all of which are known to affect dietary patterns. Other studies on defined age groups however, tend to support the overall pattern displayed in Table 6.[17]

Trends in the consumption of foods rich in fat mostly show similar patterns to the overall intake; the exception being margarine. The consumption of butter rose after de-rationing, reaching 25g in 1962. For the period 1962-1974 the average intake of butter was 20g. Since 1975, however, butter intake has gradually decreased and was 13g in 1982. Lard and compound cooking fat intake has decreased from an average of 16g 10-20 years ago to 7g in 1982. In contrast, margarine intake decreased from 21g in 1950 to 14g in 1962, and then averaged 11g until 1975. Recently, margarine intake has steadily increased and was 18g in 1982. Within the

Table 6

The Mean Energy and Fat Intakes Calculated for the United Kingdom From (1) Food Consumption Level Estimates and (2) National Food Survey 1952-1982[14]

	(1) Consumption Estimates			(2) National Food Survey		
Year	Energy[a,b] (MJ)	Fat[c] (g)	% Energy[d]	Energy[a] (MJ)	Fat[c] (g)	% Energy[d]
1952	12.7	124	36.8	10.2	94	34.5
1953	13.0	130	37.8	10.5	101	36.0
1954	13.3	138	38.9	11.0	107	36.5
1955	13.3	139	39.5	11.0	107	36.5
1956	13.3	139	39.5	11.0	108	37.1
1957	13.3	140	39.6	10.8	110	38.1
1958	13.3	141	39.9	10.9	111	38.3
1959	13.1	138	39.6	10.8	110	38.3
1960[e]	13.1	138	39.6	10.8	112	38.9
				11.0	115	39.3
1961	13.2	140	40.0	11.0	116	39.6
1962	13.3	144	40.9	11.0	117	40.0
1963	13.3	143	40.5	11.1	118	39.8
1964	13.2	144	41.0	10.9	116	40.3
1965	13.2	140	40.1	10.9	116	40.3
1966	13.2	144	41.0	10.7	117	41.0
1967	12.9	143	41.8	10.8	119	41.3
1968	12.9	144	41.9	10.7	118	41.5
1969	13.0	146	42.3	10.8	120	42.0
1970	13.0	145	42.0	10.9	121	41.8
1971	12.9	144	42.1	10.6	119	42.3
1972	12.8	143	41.9	10.2	112	41.5
1973	12.7	141	41.6	10.0	111	42.0
1974[e]	12.4	132	40.2	9.8	110	41.9
				9.7	106	41.3
1975	12.2	130	40.1	9.6	107	42.2
1976	12.2	130	40.1	9.6	105	41.7
1977	12.3	131	40.3	9.5	105	41.9
1978	12.2	130	40.0	9.5	106	42.0
1979	12.3	134	40.8	9.5	106	42.4
1980[e]	11.9	128	40.6	9.4	106	42.6
	11.7	125	40.5			
1981	11.6	126	40.9	9.3	104	42.2
1982	11.8	128	41.1	9.1	103	42.6

FOOTNOTES

a) Total food energy derived from protein, fat and carbohydrate.
b) Including alcohol.
c) Fat intake expressed in terms of grams/person/day.
d) Percentage of the total food energy derived from fat.
e) In 1960, 1974 and 1980 changes in methods resulted in two estimates.

increase in margarine intake for this period, consumption of soft margarine rose by 65% and packet margarine by only 5%.

Insofar as precise statistics are available, regional and social-class preferences in fat consumption patterns appeared to reassert themselves after de-rationing to pre-war habits.

Regional differences in total household fat intake were remarkably small during this period and no region differed from the UK average by more than 2g with the exception of Scotland which showed a figure 10g below average. In contrast, however, consumption of total butter fat (including dairy products as well as butter), in Wales, the South-West and the South-East including London was about 4g above average, East and West Midlands had average consumption, and the remaining regions had 2-4g less than average. Areas having high intake of commercially hydrogenated fat have been found to have low butter consumption.[18]

There is persisting evidence (albeit inexact) from the NFS of a higher fat intake of the upper compared with the lower classes, but the differences do not seem as great as they were in the early part of the century.[19] The 1980 NFS suggests that those in professional classes A (classification based purely on income of the head of the household - highest income class - A_1), derive 43.6% of their energy from fat compared with class C with 40.9% and class D_1 (manual workers) who derive 41.4%. There is also a gradient in butter consumption, being highest for A_1 (6g above average) and lowest for C and D_1 (2g below average). The gradient in respect of HF is in the reverse order with A_1 showing 4g less than average and C and D_1 2g higher than average.[18]

4.2 Consumption of Fatty Acids in the United Kingdom

If it is recognised that the data on total fat intake is often of rather doubtful validity, not only because of the need to rely on weighed intakes, but also because of the importance of cooking techniques and eating habits in determining fat intake, then the problem of assessing fatty acid intake is very much worse. Table 7 summarises the estimated fatty acid content of the average household, using techniques employed by the NFS.[14,20]

Table 7
Estimated Fatty Acid Content[a] of the Average Household Diet[14,20]

Year	Saturated	Monounsaturated	Polyunsaturated	P/S Ratio
1959	53.0	43.0	9.2	0.17
1969	56.7	46.5	11.0	0.19
1972	52.0	42.9	11.5	0.22
1973	51.5	41.9	11.5	0.22
1974[b]	51.4	41.2	10.8	0.21
	50.7	39.8	10.6	0.20
1975	51.7	39.8	10.1	0.19
1976	50.1	39.7	10.5	0.20
1977	47.5	39.0	10.4	0.21
1978	47.2	39.3	10.6	0.22
1979	47.8	39.7	10.7	0.22
1980	46.8	39.6	11.3	0.24
1981	45.6	38.9	11.4	0.25
1982	44.4	38.7	12.1	0.27

FOOTNOTES

a) All values expressed in g/person/day.
b) In 1974 changes in methods resulted in two estimates.

It can be seen from above that the ratio of polyunsaturated to saturated fatty acids (the P/S ratio) has altered since 1959. There has been a decline in saturated fatty acid intake and PUFA intake has increased; the two effects combined have resulted in a P/S ratio of 0.17-0.19 in 1959-1969 being increased to 0.27 in 1982.

In addition to saturated fatty acid intake being reduced, mono-unsaturated fatty acid intake was also less giving the impression that an appreciable decline in total fat intake has coincided with an increase in the proportion of fat in the diet. The explanation is that the total energy content of the diet has fallen. In other words less fat is being eaten now, on average, but it constitutes a higher proportion of the total energy relative to 20-30 years ago.

Despite the difficulties that exist in assessing the fatty acid intake of a population, it is noted that the figures are similar to those estimated by Thomas in his careful work on the percentage fatty acid composition of UK average dietary fat for the period 1960-1973 (see Part One, Section One, 6.2).

It has been suggested that measurements of the fatty acid composition of adipose tissue of randomly selected individuals within the population would provide a much better index of changes in fatty acid intake as the fatty acid profile of adipose tissue has been suggested to offer a reflection of the fatty acid composition of the diet.[21,22,23,24] These, however, are not yet available.

It must be borne in mind however that *de novo* synthesis of fatty acids in adipose tissue can occur, as can a certain amount of desaturation (see Part One, Section One, 7.2). Thus, the proportions of lower chain acids ($>C_{10}$) and certain saturated and monounsaturated acids, may give an inaccurate reflection of dietary levels. Information that such measurements could accurately provide would be PUFA intake (particularly 18:2) as it is generally accepted that the human body cannot synthesise 18:2. Any 18:2 present is therefore derived from exogenous sources.

4.3 General Summary

The results of dietary studies are readily criticised because the techniques used in collecting data are inadequate or because the sampling method may lead to unjustified assumptions about the diet of the normal population. Nevertheless, it is hard not to conclude, on the basis of the available data, that the British population have during the course of the present century, seen a very substantial increase in fat intakes once this is expressed on an energy basis. This distinction between total fat intake and the proportion of energy derived from fat is important because quite often the two values go in opposite directions. Fat intakes in g/person/day have declined over the last 10-15 years, but with the greater fall in carbohydrate intake,[14] the proportion of energy derived from fat remains high.

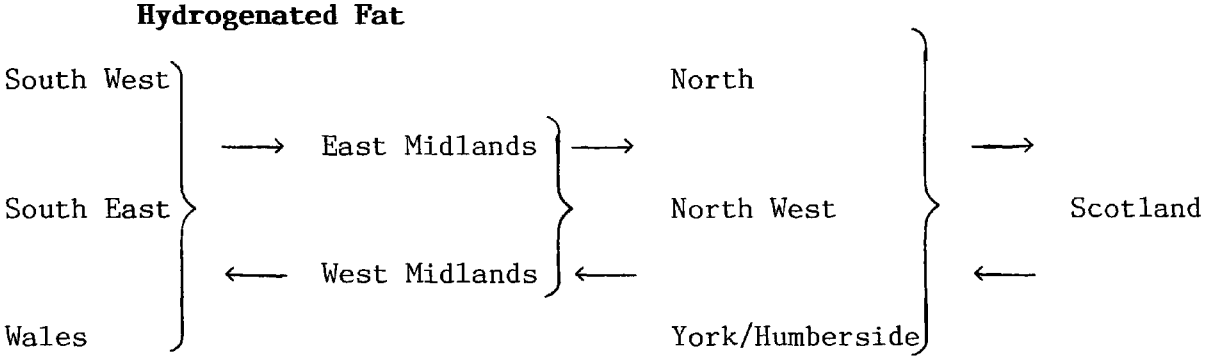
There is no physiological reason why fat consumption is as high as it is in Western society as there appears to be no absolute dietary requirement for fat except for a small amount (1-2% of energy requirements) of EFA. The prime function of visible fat is as an energy store for use by the body in times of shortage. Such a store plays an important role in the lives of certain animals who can find very little to eat during hard winter months or perhaps during long periods of drought. This may also apply to certain groups of people who may be able to eat well during certain seasons but who also have to face periods with little available to eat. Most people however, seldom have need for large stores of fat as we eat daily and it is only perhaps in a case of illness that we do not consume more than adequate amounts of food.

Social class differences are still as evident now as they were at the turn of the century and in the pre-war period with the higher social

classes consistently having a higher energy intake derived from fat. Nevertheless, whereas differences in total fat intake are small and probably of little significance, but with the higher social classes having highest consumption, the following gradients exist in respect of HF and butter fat (arrow indicates direction of increasing consumption):-

Standard Regions

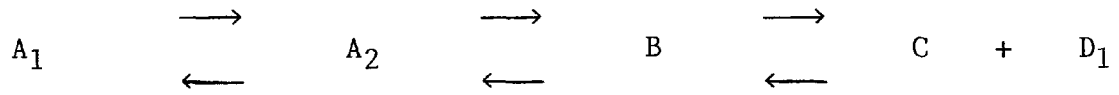
Hydrogenated Fat



Butter Fat

Social Classes

Hydrogenated Fat



Butter Fat

It is obvious from superimposing the above trends that the highest consumption of butter fat (and lowest consumption of HF) will appertain for social class A_1 in the South, South East and West and the lowest consumption of butter fat (and highest consumption of HF) in the lower classes in Northern England and Scotland.

Fatty acid intakes are even more difficult to assess than fat intake.

Nevertheless there is a suggestion that, over the last two decades, PUFA intake in absolute terms has risen and saturated fatty acid intake has fallen.

5 Hydrogenation of Fats and Oils

Most vegetable and marine oils consumed today are partially hydrogenated to obtain a more desirable physical form and improved stability when stored. Since the discovery of catalytic hydrogenation in 1897,[25] and its subsequent application to the hardening of vegetable oils, partially hydrogenated vegetable and marine oils have become a major food fat in industrial countries. Much of the motivation for hydrogenation of fats has been to provide relatively cheap, palatable and stable fats of controllable properties for mass consumption.

Hydrogenation with respect to mechanism, process techniques and conditions is comprehensively reviewed in the literature.[26,27,28] Basically however, hydrogenation means the progressive addition of hydrogen to the unsaturated double bonds in fats under the influence of a catalyst. Normal practice is to agitate the oil, pressurised under an atmosphere of hydrogen in the presence of solid nickel which acts as a catalyst and is removed after the process.

During the hydrogenation process, saturated fatty acids and a variety of "unnatural" geometric and positional isomers of unsaturated fatty acids are formed in varying amounts, depending on the initial fatty acid composition of the oils and the process conditions employed. For example, the process of hardening can be achieved not only through progressive removal of unsaturated components, but also (or instead) by conversion to isomeric *trans* material (due to the comparatively higher

melting points of *trans* than *cis* acids). In technical hydrogenation practice, addition of hydrogen (progressive saturation) is unavoidably accompanied by geometrical isomerisation, but the operating conditions may be chosen to maximise *trans* acids if required. It is in fact possible to produce a hard product with almost as much unsaturated components as the original oil. Such margarines retain high levels of unsaturation including the polyunsaturated 18:2. These margarines would be regarded by majority medical opinion as beneficial and there is thus an incentive to increase production of such types. It should be borne in mind, however, that high levels of 18:2 in such materials is obtained only through concomitant formation of appreciable amounts of *trans* isomers including isomers of 18:2 other than natural linoleic acid.

Generally however, the partial hydrogenation process reduces the PUFA content and elevates the monoene content dramatically. The remaining PUFA usually consist of a mixture of geometrical and positional diene isomers in addition to the natural isomers initially present.

The word "unnatural" is used to refer to all positional isomers of *cis* and *trans* fatty acids in which the double bond is located in a position not normally associated with the particular fat or oil in question. Some individuals who would be very distressed to see processed fats and oils labelled as containing unnatural fatty acids are quick to point out that there are enough exotic plants and unusual microorganisms that produce rare isomers to account for nearly all the possible isomers and therefore, the isomers should not be called "unnatural".[29] An unusual isomer may not be unnatural to a particular organism, but it is certainly unnatural with regard to the natural content of most fats and oils that represent a significant portion of the human diet. The quantity of any

unusual isomer produced by an organism that might find its way into the diet of humans would be insignificant compared to the quantities of the "unnatural" isomers consumed daily by most humans from partially hydrogenated fats and oils. As the quantities are usually large and the isomers were not present in the unprocessed oils, the usage of the word "unnatural" for the geometrical and positional isomers produced during the partial hydrogenation of fats and oils appears appropriate.

6 Dietary Fats and Health

6.1 General Review of the Literature of the Effects of Dietary Fats on Nutrition And Health

Worldwide, some 60 million tons of dietary lipids are consumed per year, indicating not only the importance of edible oils in world economy, but also the magnitude of their impact on world health. As a result of the increasing consumption of HF in modern diets, interest has been expressed by nutritionists and lipid biochemists in the role that unnatural fatty acid isomers play in human nutrition.

The study of the biological action of HF started in 1921 with an examination of their intestinal absorption.[30] In 1954, the first extensive investigations into the growth action of HF were reported.[31] It was found that severely hydrogenated fat as the only source of fat in the diet caused a progressive decline in the growth of rats and augmented EFA deficiency.

In the light of the increasing consumption of HF in the human diet over the past 20-30 years, it is surprising that, until comparatively recently, essentially no complete data have been available on the incorporation into human tissues of unnatural isomers present in this

source of dietary fat. Such data that are available in the literature have been summarised by Ohlrogge[32] and spans a period between 1957[33] and 1982.[34]

Opinion as to the extent and nature of the effects that unnatural fatty acid isomers have on nutrition and health in humans is polarised between the views that the presence of such isomers do not have any significant effect and are metabolised by the body in the same manner as their naturally occurring analogues; and that the presence of such isomers do, to varying extents, play important roles in human metabolism and can have adverse effects.

Most research on the impact of such dietary fats on health and nutrition have been directed towards the following questions:-

(1) Do humans incorporate into their tissues the unnatural isomers which are present in hydrogenated fats.

(2) Does human metabolism selectively recognise and metabolise these fatty acids.

(3) Do any of the unnatural isomers accumulate in human tissue.

Certainly the presence of *trans* acids in human adipose tissue[32] is evidence that such isomers are not discriminated against by the various enzymes and biochemical pathways responsible for fat absorption and its ultimate incorporation into human tissue.[35] It has been observed by some workers, however, that triacylglycerols contain higher levels of *trans* acids than phospholipids.[32] Although this observation may reflect that a greater proportion of fatty acids in phospholipids originate from *de novo* synthesis as opposed to dietary sources, it cannot be discounted that there may be discrimination against incorporation of unnatural

isomers into the phospholipid classes.

It has been suggested by several workers[21-24] that the fatty acid profile of adipose tissue reflects the fatty acid composition of the diet. Furthermore, results of long term feeding studies and measurements of turnover rate of fatty acids in humans,[23] have indicated that the adipose composition roughly reflects a 1-2 year averaging of the dietary intake. These observations allowed Ohlrogge to make tentative conclusions regarding the relative contributions of industrial versus biological hydrogenation of sources of *trans* acids in the American diet .[32] It was shown that for the subjects analysed, the major source of 18:1 *trans* isomers is HVO and that contributions from RAF were fairly minor. Furthermore, the investigation concludes that although there was a general trend toward greater accumulation of those fatty acids with the double bond farthest from the carboxyl, in no case was major accumulation of an isomer in adipose tissue observed. Thus, he concluded, that at current levels of dietary consumption, human metabolism appears able to turn over these acids at rates sufficient to prevent any major accumulation in the tissue lipids and hence cause no adverse effects.

Animal feeding experiments by several workers have led them to the conclusion that the *trans* acids have no specific atherogenic effects. Gottenbos states that they were found to be no more atherogenic than saturated fatty acids.[36] However, in humans, the effect of *trans* fatty acids on the serum cholesterol level was found to be intermediate between that induced by saturated and *cis* fatty acids. Kritchevsky concluded that the data currently available suggest that there is no special deleterious role for *trans* unsaturated fatty acids and that

their metabolic effects resemble those of saturated fat.[37]

The above views are not universally accepted and there are those who would argue that the possible influence of not only *trans* but in addition unnatural *cis* fatty acids on heart disease, cell metabolism and cell structure is still a matter for debate.[38] Kummerow has stated that the *trans* fatty acids in dietary fats may influence the rate of oxidation of substrate in heart mitochondria, the synthesis of prostaglandins and the fluidity of the lipid phase in cell membranes.[38,39] However, whether any or all of these observations are significant to the development of heart disease he concludes is a matter for further research.

Kummerows' observations are confirmed by the work of Holman[40] who has studied the influence of HF on the metabolism of PUFA. The following conclusions were drawn.

(1) Isomers of 18:1 *cis* in which the double bond occurs close to the carboxyl are not well hydrolysed by pancreatic lipase.

(2) Rates of catabolic oxidation of the 18:1 isomers are dependent upon positional and geometric structure.

(3) Both the position and geometry of unsaturation in 18:1 influence the inhibition of desaturase reactions critical to the metabolism of PUFAs.

(4) The presence of such unnatural fatty acid isomers may compete with common substrate fatty acids critical to PUFA metabolism giving rise to a whole range of PUFAs of unusual structure. The metabolic effects of such unusual prostaglandin precursors cannot be predicted.

Overall, he concludes that each isomer is a unique substance with its own rate of metabolism at each step, leading to different products which also must have unique biological properties. Thus, each isomer must be recognised as an entity because biological systems do just that.

Recently, epidemiological studies have revealed a highly significant correlation between dietary fat intake and certain forms of cancer.[41] The concept that a diet rich in PUFA could be associated with increased susceptibility to cancer was also given impetus by a report that in an extensive therapeutic trial of the effect of PUFA on coronary heart disease, the treated group exhibited a greater death rate because of various forms of cancer.[42] Although another report revealed that in other trials no such trend was seen,[43] the earlier report received considerable attention. Smith concludes in his review that at the present time diets rich in PUFA are more effective than diets rich in saturated fat in enhancing tumorigenesis in animals.[44]

6.2 Dietary Fat and Ischaemic Heart Disease

The underlying feature of most heart disease is atherosclerosis, an irregular thickening of the inner wall of the arteries which reduces the size of the lumen of the vessel. When this occurs in the arteries that provide nutriment for the heart muscle, the disease is called coronary heart disease. It has been called angina pectoris, disease of the coronary arteries, arteriosclerotic heart disease and is now called ischaemic heart disease (IHD). In addition to factors such as stress, smoking, high blood pressure and hereditament, the types and levels of dietary fats have also been implicated in the causation of IHD.

The desirable amount and types of fat in the diets of individuals have

been the subjects of considerable controversy. On health grounds, medical opinion has tended towards the view that total fat consumption should be reduced and further, that consumption of saturated animal fats should be reduced and replaced in some measure by unsaturated vegetable fats including margarine. More recently , the accent has been on substitution by polyunsaturated fats which usually (but not invariably) are present in softer margarines in substantial amount.

Such recommendations are based on the generally accepted view that saturated fats have a serum-cholesterol level elevating effect and polyunsaturated fats, a serum-cholesterol level lowering effect. Groups that have made such recommendations include the American Heart Association,[45] the US Senate Select Committee on Nutrition and Human Needs[46] and the US Department of Agriculture and Department of Health, Education and Welfare.[47]

In the UK, the recent James (NACNE) report has set nutritional targets in dietary fat intake in the UK.[48] The James report recommends:

(1) 10% reduction in fat intake;

(2) 15% reduction in saturated fatty acids;

(3)in order to ease the reduction of saturated fatty acids an increase in PUFA intake of 25%.

The subsequent DHSS (COMA) report[49] although following the trend recommended more drastic reductions of:

(1) 17% reduction in fat intake; and

(2) 25% reduction in saturated fatty acids.

The recent emphasis on substitution by polyunsaturated fat is prompted by statistics such as those from the US which indicate that the death

rate because of IHD in the US between 1968 and 1976 fell by 21%. In the UK for the same period, mortality rates for heart disease have remained constant. Statistics indicate during this period a steady increase in the percentage of linoleic acid in the adipose tissue of Americans. In contrast, the linoleic acid content of the adipose tissue of the British population does not seem to have changed. The data thus suggests that the decline in IHD mortality in the US was preceded by an increase in the consumption of polyunsaturated fat. It has been noted that, although these data do not prove a causal relation between dietary fat type and IHD, they do fit the different mortality trends in the US and UK remarkably well.[50]

The dangers of international comparisons of death rates from IHD have however been expressed.[51] In the American context it is true that consumption of vegetable oils and PUFA can be boosted by substitution of animal fat by margarine. In the UK context however, because of the diverse nature of fats and oils used in margarine production, the result of such substitution may well be very different; there may in fact be an increase in saturated fat and a decrease in PUFA intake. In view of such dissimilarities in UK and American margarines, it is considered that "extrapolation" of American claims in the "butter versus margarine" controversy could be a dangerous manoeuvre. The view should be taken therefore that international epidemiological comparisons are meaningful only when comparing like with like.

The contrary view that there may be hazards associated with the altered fatty acid components of industrially-hydrogenated oils and fats has from time to time been reported in the literature.

Despite the difficulties and dangers (as indicated above) in international comparisons of death rates from IHD, it has been pointed out that in Europe for example, Southern Europeans such as the Spaniards, Italians, Yugoslavs and Greeks, who consume little HF have considerably lower death rates than Northern Europeans like the British, Germans, Swedes and Finns who consume large amounts of HF.[52] Eastern Europeans like the Bulgarians and Romanians who consume large amounts of saturated fats and cholesterol have low death rates from IHD; only trace amounts of *trans* acids were found to be present in their red blood cells whereas levels in samples from Finland and America were reported to be 2.6% and 2.0% respectively.[39]

On purely epidemiological grounds, Thomas reported in 1975 that for the UK, mortality from IHD is highest in those areas and those social classes which consume the highest amounts of industrially hydrogenated fat and lowest amounts of total butter fat.[18]

In view of this evidence, it was decided to set up a further study of dietary fat and IHD on a more direct and "experimental" basis by examination of the composition of the body depot fat of persons dying of IHD compared with the body depot fat of persons dying of unrelated causes. Since this work initiated the work described in this thesis, it will now be summarised in some detail.

Initially, to ascertain whether there are present in the UK fat diet, certain recognisable features which would act as markers, the following characteristics of British dietary fats were determined.[53]

British HF contains high quantities of firstly geometrical (*trans*) (T) isomers which are derived from hydrogenation of both marine and

vegetable oils, and secondly "higher acids" (H), C_{20} and C_{22}, mainly monoenoic acids (themselves largely in the *trans* configuration) with small amounts of saturated, di- and trienoic components which derive from HMO. Such acids are largely, but not entirely, absent in natural fats. (RAF contains 5-6% T acids as a result of bacterial hydrogenation in the rumen - levels substantially lower than in industrially hydrogenated materials. It must be borne in mind however that consumption of RAF in Britain is almost three times greater than that of HF, so its contribution to total *trans* acids, T, is not negligible - ca.2.1%. Contributions from HF to T is calculated as 3.3% thus giving a value of 5.4% for T in average UK dietary fat).

Unlike T and H which in the main reflect HF consumption, a third parameter is much more characteristic of dairy fat intake, namely "lower acids" (L) which are defined as the sum of acids 14:1, 15:0, 15:0br, 16:0br, 17:0 and 17:1. These are present in higher amounts in RAF such as butter than in HF (ca. 2.2%), and are virtually absent in all vegetable oils and fats (0.3%). 0.3% Of L are also derived from pig and poultry fat. Thus the proportion of L in UK average fat was estimated at 2.8%.

Proportions of L and H were determined by packed column GLC by use of two liquid phases (EGSS-X and EGSS-Y) of differing polarity and total *trans* fatty acids (T) were determined by infrared spectroscopy in the same manner as were used for the analysis of adipose tissue. The distribution of these "diagnostic" acids in the UK diet are summarised in Table 8.[53]

Details for margarines were based on analysis of seven (leading) retail

brands of margarines purchased in 1976; those for cooking/frying oils on four readily available retail brands and on oils extracted from potato crisps.

Table 8
Distribution of T, H and L in UK Dietary Fats[53]

Materials	%T	%H	%L	Ratio T/L
Average hard margarine	30	12	2	15
Average soft margarine	12	4	0.2	60
Branded shortenings	34	18	3	11
Cooking oils (variable)	5	1	0.1	50
Butter fat	7	2	5	1.4
Beef fat	5	2	8	0.6
Mutton fat	6	3	5	1.2
Lard	0	2	1	0

Hard margarines varied considerably in their T and H content (10-40% and 8-16% respectively), amounts which reflect mainly respective contents of HMO (10-70%). Soft margarines too had very variable T content (6-12%), but this arises from differing amounts of HVO hydrogenated to varying degrees. Tabulated figures for "average" hard and "average" soft UK retail margarines were calculated by weighting the brands according to market shares.

The tabulated data for butter fat are based on analysis of three popular brands purchased in 1976 and six similar brands purchased in 1980 which had T contents varying between 7 and 8%. It is difficult to know whether such levels truly reflect average UK butter fat, but the figures obtained by Thomas et al. are higher than those found by Hay and Morrison[54] (3-6% according to season) in their work on British milk fat.

The most probable figure for T was thought to be between 5 and 6%.

Ambiguities in the use of T and L in isolation as markers may be circumvented by the employment of the ratio T/L and as such, the ratio was central to the investigation.[55] Table 8 indicates that, whereas this ratio is near unity in RAF, it is about 15 for HMO and even higher for HVO. Since all other fats do not contribute to any great extent, an increase in HF in a fat mixture relative to the amount of RAF will unambiguously result in an increase in the value of ratio T/L.

Many workers have suggested that the fatty acid profile of adipose tissue reflects the fatty acid composition of the diet.[21-24] However, a difficulty in holding the opinion that depot fat composition reflects only that of the fat content of the diet would appear to be that *de novo* synthesis of fatty acids from glucose in adipose tissue can undoubtedly occur. The view further appears to be that such conversion will be into palmitic acid, thereby resulting in depot fat high in C_{16} content. Nevertheless, it has been noted that conversion of carbohydrate into fat becomes less important as the proportion of fat in the diet is increased and that samples of human adipose tissue - taken from Western subjects with their high fat diet - indicate that little or no *de novo* synthesis of fatty acids occurs.[56]

Except possibly under conditions of severe fat deprivation, it may be concluded that amounts of endogenous fat deposited are negligible in normal healthy man compared with deposits of exogenous origin; the composition of depot fat may then only reflect that of the dietary fat. The subject is thoroughly reviewed by Winter.[24]

The difficulty of ensuring compliance of an individual with a given

fatty diet, may be overcome by a comparison of a population-average depot fat composition with its average fatty diet. Although details of the calculation are not given here, the fatty acid composition of such a diet for C_{14} acids upwards as calculated by Thomas is summarised in Table 9. This was compared with the average composition of 95 control samples of adipose tissue drawn from various regions of England and Wales, the result of which are also summarised in Table 9.[53]

Bearing in mind the difficulties involved, agreement was very satisfactory. Dietary levels of 16:0 and particularly of 18:0 were found to be higher than depot fat percentages. Combined quantities (16:0 plus 16:1 and 18:0 plus 18:1) however, were seen to agree closely. It is seen too that agreement extended to such minor components as odd and branched-chain acids (L) and acids higher than C_{18} (H). The average value of T in the depot fat samples was 5.2% compared with 5.4% estimated for the average diet.

Although there is no easy way of demonstrating that the premise upholds for any given individual, it was proposed, for such tissue samples which formed the subject of the investigation, that a population average depot fat composition truly reflected the fatty acid composition of its dietary fat, subject only to the difference that a measure of desaturation of dietary 16:0 to 16:1 and 18:0 to 18:1 occurred. In particular, the view was held that a group of persons consuming margarine at the expense of butter would show corresponding changes in T, H and L.

On the above premise, a programme of work was commenced in 1975 using specimens of adipose tissue supplied through the courtesy of Dr P C

Table 9

The Percentage Fatty Acid Composition of the Average UK Dietary Fat and Average Control Adipose Tissue[53]

Fatty Acid	Dietary Fat		Adipose Tissue Fat	
14:0	5.9		3.8	
15:0,15:0br,14:1	1.4		1.3	
15:1,16:0br	0.2		0.2	
16:0	25.3	29.2	22.3	29.3
16:1	3.9		7.0	
17:0br	0.4		0.6	
17:0	0.8		0.5	
17:1	0.4		0.7	
18:0	12.3	48.3	5.1	50.4
18:1	36.0		45.3	
18:2	8.6		8.3	
18:3	1.0		0.7	
20:0	0.4		0.6	
20:1	1.2		0.2	
20:2,20:3	0.9		0.7	
22:0	0.3		0.1	
22:1	0.9		0.6	
20:4	0.1		0.2	
Total	100.0		100.0	
T	5.4		5.2	
H	3.7		4.0	
L	2.8		2.7	

Elwood, Director of MRC Epidemiology Unit, Cardiff. A total of 231 post-mortem specimens (from 10 areas of England and Wales) of adipose tissue taken from the anterior wall near to the umbilicus were analysed. 136 Specimens derived from subjects who had died of IHD (cases), the remainder being controls. Of these, the social class status of 115 cases and 76 controls were known. All were males who showed no evidence of wasting and deaths from malignant neoplasms and cerebro-vascular disease (stroke) were excluded.

The results showed that case mean values of L were lower than corresponding values for controls in 9 of the 10 areas. On average values of T were higher for the cases in 9 of the 10 areas as were values for the ratio T/L. There were no consistent differences in H or 18:2 values.[53]

After appropriate statistical analysis to match for area of residence it was shown that whereas on average T was higher in the cases (but not significantly so - 5.4% (cases) compared with 5.2% (controls)), L was significantly higher for the controls; the weighted mean value of T/L was significantly higher for the cases. Such a result was interpreted on the basis that the cases had consumed a higher proportion of HF and a lower proportion of RAF than had the controls.[55]

It was further observed that the general pattern of regional variation in ratio T/L in the controls also broadly reflected known dietary patterns. That is, in areas where HF consumption is lowest (and butter fat is highest), T/L is lowest and conversely, where HF consumption is highest (and butter fat lowest), T/L is highest.

Although it was found that the proportion of 18:2 varied between 3.7%

and 15.8%, the weighted mean value for cases is virtually identical with that for the controls. The result lends no support to the currently popular view that high amounts of polyunsaturated fats (essentially 18:2) have a beneficial effect.

Mean values of H were found to be virtually identical for cases and controls thus presenting no evidence that higher acids at the levels consumed in the UK are harmful.

In a later paper,[57] it was assumed that this common value of H implied a common mean value of T_H and therefore that "higher *trans*" acids carried no hazard. This led to an examination of case versus control values of T/L at comparable values of H in the belief that any difference would then be because of differences in the T_L content. It was in fact shown that on such basis, the mean value for cases was significantly higher than that of the controls. It was thus concluded that risk attaches only to lower *trans* acids i.e. to the 18:1 *trans* plus 16:1 *trans* content of fats.

With the development of highly polar cyanosilicones in the mid 70s, it became possible to determine *trans* components directly by GLC. Accordingly, the above 231 specimens were re-analysed by the use of a 40'x1/8" column packed with 20% OV-275 coated on 100/120 mesh acid-washed and silanised Chromosorb P. The new set of data together with the previous information from the less polar columns enabled Thomas et al. to evaluate 16:1 *trans* and 18:1 *trans* separately. The sum of these two "lower *trans*" acids (T_L) fell short of total *trans* acids T, the difference (T_H) resulting from large amounts of "higher *trans*" acids derived from HMO.[58]

In addition UK dietary fats were re-analysed and the proportions of T_L, T_H and total T present are summarised in Table 10.[59,60]

Table 10

The Average Proportions of *trans* Components in UK Hydrogenated Materials

Material	% *trans* Acids			
	16:1	18:1	T_H	Total (T)
Hydrogenated vegetable oil	0	16	2*	16
Hydrogenated marine oil	8	10	28	46
Ruminant animal fat	<0.2	5-7	trace	5-7
Lard	0	0	0	0
Unprocessed vegetable oil	0	0	0	0

* Present in peanut and rapeseed oil

It is evident that the distribution of these two groups, T_L and T_H, differs between natural and commercially hydrogenated fat. RAF contains between 5% and 7% of T_L and low amounts of T_H. Hydrogenated vegetable oils have virtually no T_H content (with the exception of rapeseed and peanut oils) and very variable amounts of T_L (ca 12% to 30%). Marine oils are of diverse origin and the hydrogenated products contain between about 25% to 55% total *trans* (T) content. In all HMO only the smaller part of their total *trans* content is within the 16:1 and 18:1 components, the remainder being present as T_H (ca 60%). Of particular relevance in the context of the investigation was the fact that analysis of seven leading brand margarines of high HMO content showed that the ratio 16:1 *trans*/T_H was highly variable and thus amounts of 16:1 *trans* varied from margarine to margarine by a factor of about three times. The ratio 18:1 *trans*/T_H is less variable.

Most significant to note is that the "discriminating power" of 16:1

trans, unlike 18:1 *trans*, is high. It is evident from above that by far the main source of 16:1 *trans* is HMO. High levels of 16:1 *trans* are therefore almost certain to result from the presence of HMO.

Analysis of the case and control specimens resulted in the following observations. The weighted mean value of T_H for the cases, as anticipated, was virtually identical with that of the controls. In contrast, case mean percentages of 16:1 *trans* were higher than the controls in 8 out of 10 areas. Statistical analysis revealed that area to area differences were highly significant but that there was no evidence that case/control differences varied between areas. Case mean values of 18:1 *trans* were likewise higher for the controls in 8 out of 10 areas though far short of being significantly different.[56]

These results may be interpreted in one of two ways. Firstly, the most obvious interpretation is that, whereas there is no evidence that "higher *trans*" acids are harmful, risk attaches to "lower *trans*" acids and in particular, 16:1 *trans*. On this basis, HVO would appear to carry no hazard and HMO is the sole culprit. Secondly, and more likely, whereas 16:1 *trans* derives almost exclusively from HMO, only about 40% of the 18:1 *trans* acid is contributed by HF (marine plus vegetable) the remainder coming from RAF. In other words, whereas 16:1 *trans* is a sensitive measure of HMO, 18:1 *trans* is a poor "marker" of HF.

Furthermore, the implication is that risk attaches to "lower *trans*" acids (particularly 16:1 *trans*) purely in virtue of this having a *trans* configuration irrespective of other structural considerations. Whereas this is undoubtedly a possibility, it ignores the fact that *trans* acids from differing fat sources are not identical in structure.

It must be borne in mind that the process of hydrogenation gives rise to, not only geometrical isomerism, but in addition a variety of positional isomers of both *cis* and *trans* configuration which can form a substantial proportion of the whole product in both HMO and HVO. In RAF, the *trans* double bonds are more centrally situated, and randomisation of *cis* positional isomerism is far less marked.

Necessarily therefore, the 16:1/18:1 *trans* acid content of HF is not only diverse in nature but is positively correlated with amounts of positional *cis* isomers. In RAF the proprtions of the latter will be very small in comparison.

The inference that HMO carries a higher risk than HVO, the latter containing much 18:1 *trans* but no 16:1 *trans*, is considered premature in the absence of precise information as to the amounts of positional isomers and additives associated with these two types of HF. Furthermore, since in principle 16:1 *trans* and 18:1 *trans* are strongly correlated, it was considered imprudent to apportion risk to one acid more than to the others.

In addition, the ratio T_L/L was measured and was found to be significantly higher in cases in 9 out of 10 areas. The higher values of the ratio tended generally to be exhibited by areas of highest IHD mortality.

On the premise that population mean levels of "lower *trans*" and L acids in adipose tissue reflect dietary intakes, it was concluded that the cases consumed a higher amount of HF relative to RAF than did the controls. Furthermore, it was concluded that those HF having the higher content of "lower *trans*" acids present the greater risk.[58]

It should be mentioned at this point that this work is not a case/control study in the strictest sense. That is, with controls set to have zero consumption and cases consuming appreciable quantities of HF. Indeed it is most probable that, except for the occasional individual, differences in consumption are not likely to be large on account of the fact that, on average, about 40% of HF is "hidden" in the sense that it derives from such consumed items as cakes, biscuits etc bought as such. No more can thus be done other than to accept what differences may exist.

Furthermore, whereas control specimens will be expected to randomly reflect the various component fatty acids of the British diet, the case specimens will show elevated levels of firstly, particular fatty acids carrying a hazard (if any) purely in virtue of their chemical structure. Secondly, any fatty acid types which may be correlated with a toxic material (if any) purely because they occur together in the diet. That is, margarine with its high T content in addition contains for example a variety of artificial anti-oxidants which are absent in natural fats. Thus, a toxic anti-oxidant may in that case be accompanied by *trans* acids which may not themselves carry any hazard.

Finally, arising from the very nature of IHD (a large proportion suffering fatal attack with no previous overt symptoms) an unknown fraction of these decadents whose deaths were accidental and therefore qualified as controls could well have experienced fatal heart attack a short time later.

In an attempt to circumvent this latter problem, more recently, a living "case" versus control programme has been undertaken where subjects with

certain ECG evidence of ischaemia but no history of angina or infarction (cases) have been compared with controls.[60] Specimens of their depot fat were taken at biopsy and the fatty acid compostions thereof compared. The results obtained compared favourably with those of the previous investigations as it was found that whereas the proportions of certain *trans* acids characteristic of HF are higher for the cases, proprtions of L are higher for the controls. The ratio T/L was on average higher by an amount which was statistically significant. As in the previous studies, it was found that there was no significant difference between the case and control means of the percentage of linoleic acid.

The ratio 16:1 *trans*/L was again found to be significantly higher for the cases and it was thus concluded that there was good evidence that they consumed a higher amount of those margarines based on HMO relative to RAF than did the controls. The ratio 18:1 *trans*/L was also higher for the cases but again, not significantly so.[60] Once again, the arguments presented above in this Section must be borne in mind.

7 Aim of the Investigation

The natural progression of the above studies is the development of a satisfactory technique for the quantification of geometrical and positional fatty acids of adipose tissue. The infrared technique employed by Thomas permits quantification of only a total *trans* figure composed of C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids including double bond positional isomers. Subsequent GLC studies using the highly polar OV-275 packed column permitted only direct measurement of the proportions of "lower" *trans* acids (16:1 plus 18:1) together with the "higher" *trans* acids (C_{20} plus C_{22}) in addition to the measurement of the proportions

of *cis* isomers. Individual positional *cis* and *trans* acids which, on the premise that the fatty acid profile of adipose tissue reflects dietary fat consumption, are invariably present, are not resolved.

Such a task presents formidable analytical difficulties and required the synthesis of a series of positional and geometrical fatty acids as standards, and their characterisation prior to the commencement of a case versus control investigation of the levels of such acids in human adipose tissue. The work thus described in this thesis is the synthesis of a series of these acids and their spectroscopic and chromatographic characterisation. A brief literature review of synthetic methods employed in the preparation of fatty acids now follows. Literature relating to the characterisation of fatty acids are cited where appropriate in Part Two, Results and Discussion.

SECTION TWO

LITERATURE REVIEW OF SYNTHETIC METHODS EMPLOYED FOR THE PREPARATION OF FATTY ACIDS

There appear in the literature many procedures which, on paper at least, could be applied to the synthesis of unsaturated fatty acids. Such methods may be generally subdivided into two broad groups namely the chemical modification of readily available acids - usually of natural origin, and methods involving chain extension by condensation of shorter chain intermediates.

8 Modification of Naturally Occurring or Readily Accessible and Closely Related Compounds

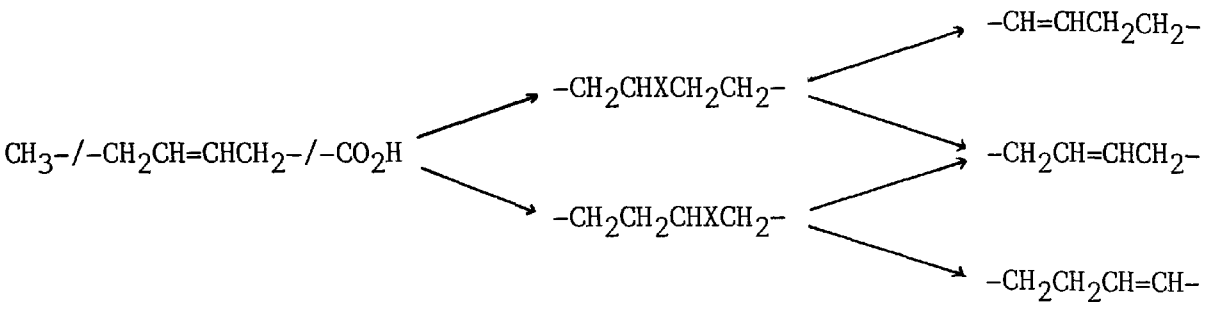
Attempts to modify readily available acids to produce unnatural compounds have not always been successful because of lack of specificity in the reactions involved and because of the difficulty in isolating individual compounds. Modification of naturally occurring fatty acids may be divided into three types depending on whether there is an increase, decrease or no change in the degree of unsaturation.

8.1 Methods Involving an Increase in the Degree of Unsaturation

The commonest method of introducing an unsaturated linkage involves dehydrohalogenation of a halogeno-compound. This reaction is generally effected by treatment with alkali or a nitrogenous base such as quinoline. Alkali, although used extensively, has the disadvantage that the product may be accompanied by some hydroxy-acid and by some isomeric unsaturated acids resulting from double-bond migration.

The halogenated acids may be produced in four ways one of which is the interaction of a double bond with hydrogen bromide or bromine. Although not in the strictest sense a method involving an increase in the degree of unsaturation, unsaturated acids yield isomeric acids by hydrohalogenation and subsequent dehydrohalogenation.

On a purely random basis, such a reaction would lead to three products:



X=Halogen

In practice however, one isomer may predominate and be isolatable. Factors affecting the product of this reaction include the position of unsaturation relative to the carboxyl group and the experimental conditions. Several octadecenoic acids have been prepared in this way.[61]

8.2 Methods Involving a Decrease in the Degree of Unsaturation

Unsaturated acids may be prepared by partial reduction of highly polyunsaturated compounds. Such reductions however generally yield a complex mixture of isomers, for, in addition to the fact that different alkenoic bonds may become saturated, *cis/trans* isomerism and double bond migration may occur simultaneously. Such reactions, although of considerable interest from other viewpoints, are of little value for preparative purposes.

However, the synthesis of unsaturated fatty acids from the non-catalytic reduction of highly unsaturated fatty acids with hydrazine has been reported in the literature.[62] The method has been used by Sebedio and Ackman to obtain C_{20} isomeric *cis* unsaturated fatty acids from 20:5(n-3).[63] A 32 component mixture was obtained which was composed of saturated C_{20} acid, 5 monoenes, 10 dienes, 10 trienes, 5 tetraenes and unreacted 20:5.

This method circumvents the problem of *cis/trans* isomerism and double bond migration. The method is not however suitable for the large scale preparation of unsaturated fatty acid isomers firstly, because pure highly unsaturated fatty acids are not readily available in large quantities and secondly, because of the difficulty in isolating individual isomers.

8.3 Methods Involving No Change in the Degree of Unsaturation

Methods that involve no change in the degree of unsaturation may be classed as those that involve stereomutation and those that involve double bond migration.

a) Stereomutation

Stereomutation of the *cis* alkenoic acids to all *trans* isomers is possible and the technique has been used by several workers.

The conversion of oleic to elaidic acid has been studied by Griffiths and Hilditch,[64] and Kircher.[65] Strong and co-workers have synthesised *trans*-11-octadecenoic acid (*trans*-vaccenic) from its *cis* analogue by isomerisation with selenium at 180–200°C in 34% yield.[66] Stereomutation has more recently been employed by Litchfield et al.,[67] and Gunstone and Jacobsberg[68] to obtain geometrical isomers of linoleic acid, and by Gunstone and Ismail in their synthesis of *trans*-octadecenoic acids.[69]

Suitable reagents other than selenium for stereomutation include nitrous acid, several sulphur containing compounds e.g. $HSCH_2CH_2CO_2H$, $HSCH_2CH_2NH_2$ and iodine and/or ultra-violet light.

Stereomutation is unavoidably accompanied with double bond migration. Additionally, the process is an equilibrium reaction resulting in the

formation of a *cis/trans* mixture. The combined result of these phenomena is a crude product, the melting point of which is considerably lower than that of the purified product, which may be isolated by repeated crystallisation or by argentation chromatography.

Stereomutation is also known to occur under conditions of hydrogenation and also during autoxidation.[26,70]

b) Double Bond Migration

The double bond(s) present in an unsaturated fatty acid may migrate under certain conditions. Double bond migration may be acid or base catalysed and tends to yield the most stable double bond isomer. That is, one in which the double bond is conjugated or most heavily substituted.[71]

Generally, in monounsaturated fatty acids, the double bond migrates towards the carboxyl end of the molecule. Farmer states that under the appropriate conditions, oleic acid affords a variety of isomeric octadecenoic acids.[72] Such migrations are unavoidably accompanied by stereomutation. Double bond migration, as with stereomutation, is also known to occur under conditions of hydrogenation.[26]

The technique of double bond migration is however little used for the preparation of fatty acids as competition from addition, polymerisation and skeletal rearrangements limits the use of the method.

9 Methods Involving Chain Extension and Condensation Reactions

Several condensation reactions have been utilised in the preparation of unsaturated fatty acids. The double bond may be formed at the point of condensation or, alternatively the double bond or some suitable pre-

cursor may be present in one of the reacting components. These methods may be further divided into three types according to the number of carbon atoms added to the starting material.

9.1 Reactions Resulting in the Addition of One Carbon Atom

Of the standard procedures used for increasing the length of a chain by one carbon atom, that involving the reaction sequence given below has been used most extensively.

$$RCO_2H \xrightarrow{R'OH} RCO_2R' \xrightarrow{LiAlH_4} RCH_2OH \xrightarrow{SOCl_2} RCH_2Cl \xrightarrow{NaCN} RCH_2CN \xrightarrow{OH^-} RCH_2CO_2H$$

Many steps are involved but yields are good and pure products are obtained given pure starting materials.

10-Undecenoic acid has been converted into 11-dodecenoic acid[73] and the method has since been used in a number of applications for the synthesis of acids, most invariably in combination with other methods.

9.2 Reactions Resulting in the Addition of Two Carbon Atoms

By using diethyl malonate it is possible to add two carbon atoms to an appropriate alkyl halide. Subsequent alkaline hydrolysis and decarboxylation result in the desired acid.

$$R\text{-}Cl + \begin{array}{l} CO_2Et \\ | \\ CH_2 \\ | \\ CO_2Et \end{array} \xrightarrow{NaOEt} \begin{array}{l} CO_2Et \\ | \\ R\text{-}CH \\ | \\ CO_2Et \end{array} \xrightarrow[(2)H^+/-CO_2]{(1)OH^-} R\text{-}CH_2CO_2H$$

The method has been applied to the synthesis of unsaturated fatty acids. Klok and co-workers have reported the synthesis of 15-octadecynoic acid, 17-eicosynoic acid and 19-docosynoic acid from the repeated malonic ester synthesis from 13-hexadecyn-1-ol in accordance with the scheme

outlined below. Subsequent partial reduction of the acetylenic bond yielded the *cis* acid in 33.8% yield.[74]

$$CH_3CH_2C{\equiv}C(CH_2)_{12}OH \xrightarrow{MeSO_2Cl} CH_3CH_2C{\equiv}C(CH_2)_{12}OSO_2Me \xrightarrow[(2)OH^-\quad(3)H^+/-CO_2]{(1)NaOEt/CH_2(CO_2Et)_2}$$

$$CH_3CH_2C{\equiv}C(CH_2)_{13}COOH \xrightarrow{LiAlH_4} CH_3CH_2C{\equiv}C(CH_2)_{14}OH \longrightarrow$$

15-octadecynoic acid

$$CH_3CH_2C{\equiv}C(CH_2)_{15}COOH \longrightarrow CH_3CH_2C{\equiv}C(CH_2)_{16}OH \longrightarrow$$

17-eicosynoic acid

$$CH_3CH_2C{\equiv}C(CH_2)_{17}COOH$$

19-docosynoic acid

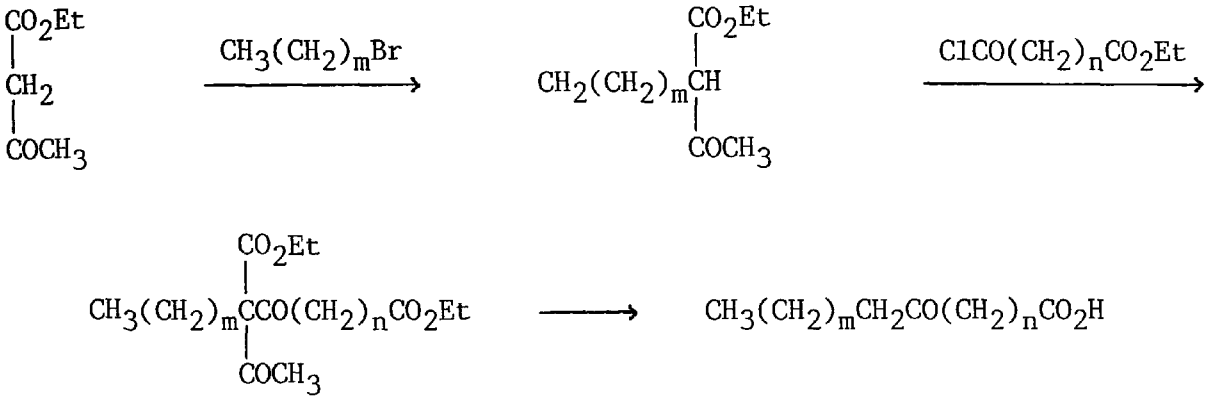
Another method involves condensation of an aldehyde with malonic acid, generally in the presence of pyridine, which after subsequent decarboxylation yields a *trans*-2-unsaturated acid.[75,76]

$$CH_3(CH_2)_nCHO + \begin{matrix} CO_2H \\ | \\ CH_2 \\ | \\ CO_2H \end{matrix} \longrightarrow CH_3(CH_2)_nCH{=}C\begin{matrix} CO_2H \\ | \\ | \\ | \\ CO_2H \end{matrix} \longrightarrow CH_3(CH_2)_nCH{=}CHCO_2H$$

9.3 Reactions Resulting in the Addition of Several Carbon Atoms

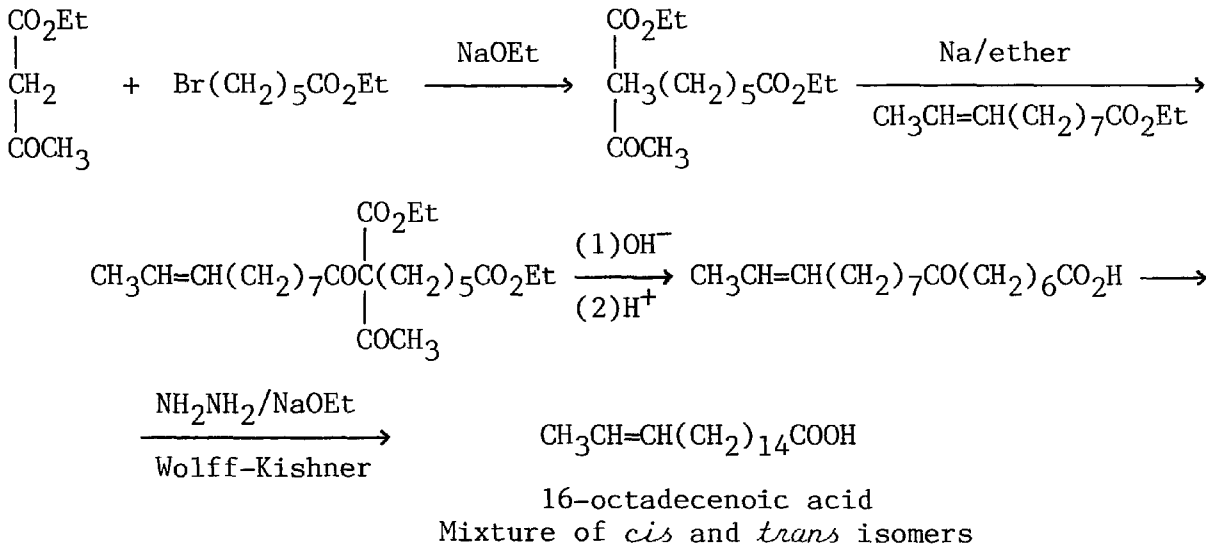
a) The Use of an Acetoacetic Ester as a Coupling Unit

In 1925 Robinson and Robinson described a method of preparing long chain keto-acids involving condensation of an alkyl halide and a carbethoxy-acyl halide with an acetoacetic ester. Subsequent stepwise hydrolysis afforded a keto-acid which may be reduced by either the Clemmensen or Wolff-Kishner reaction to yield the saturated acid.[77]



Subsequently, one of the authors drew attention to the rather low yields obtained.[78] The method was subsequently improved and has been used for the synthesis of several acids. It is limited only by the accessibility of the α,ω-bromoesters.

The method was subsequently adapted to the synthesis of unsaturated fatty acids. The synthesis of 16-octadecenoic acid by Kapp and Knoll is typical.[79]



b) Condensation Involving Organo-metallic Compounds

In 1934, Noller and Bannerot described the first synthesis of oleic and elaidic acids. The main reaction was the condensation of 1,2-dibromo-9-

chloro-1-methoxynonane with octylmagnesium bromide yielding 8-bromo-1-chloro-9-methoxyheptadecane.[80] Removal of the 8,9- substituents furnished a double bond in this position and the chloride was converted into the acid via the nitrile. The resulting product was a mixture of *cis* and *trans* isomers of 9-octadecenoic acid.

$$Cl(CH_2)_7CH_2CHO \xrightarrow[(2)MeOH/HBr]{(1)Br_2} Cl(CH_2)_7CHBrCHBrOMe \xrightarrow{BrMg(CH_2)_7CH_3}$$

$$Cl(CH_2)_7CHBrCH(OMe)(CH_2)_7CH_3 \xrightarrow[(3)OH^-]{(1)Zn\ (2)NaCN} HOOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$$

9-octadecenoic acid
Mixture of *cis/trans* isomers

The disadvantage of this method is that the starting materials, the α,ω-chloro aldehydes, are difficult to obtain.

Modifications of this process have resulted in the preparation of elaidic and *trans*-vaccenic acids.[81] In these preparations, the double bond is already present in one of the starting materials.

$$CH_2{=}CHCH_2(CH_2)_nCO_2Me \xrightarrow{C_2H_4(CO_2)_2NBr} CH_2{=}CHCHBr(CH_2)_nCO_2Me \rightleftharpoons$$

$$BrCH_2CH{=}CH(CH_2)_nCO_2Me \xrightarrow{CH_3(CH_2)_mMgBr} CH_3(CH_2)_mCH{=}CH(CH_2)_nCO_2Me$$

The products in both cases were found to be contaminated with their *cis* isomers and with a small amount of hexadecenoic acid.

More recently, a procedure based on carbon-carbon bond formation by selective coupling between one alkyl group of a "mixed" copper(I)ate complex and primary iodoalkyl carboxylic esters has been applied to the synthesis of fatty acids.[82] It is claimed that the procedure is

compatible with a number of functional groups, yields products cleanly and in high yield, and is applicable to a number of classes of fatty acids. The method is applied to the synthesis of unsaturated acids by the use of an unsaturated Grignard reagent. A typical procedure, that for ethyl 21-docosenoate, is outlined below.

$$CH_2{=}CH(CH_2)_8CH_2Cl \xrightarrow[(2)CH_3Cu(I)]{(1)Mg/THF} CH_2{=}CH(CH_2)_8CH_2^{-}CuCH_3MgCl^{+} \xrightarrow[THF]{I(CH_2)_{10}CO_2Et}$$

$$\longrightarrow \quad CH_2{=}CH(CH_2)_{19}CO_2Et$$

79%

The reaction has the advantage that it involves only one coupling step. Its disadvantages are that the synthesis of unsaturated fatty acids results in the formation of *cis* and *trans* isomers and the ethyl iodoalkanoates are not readily accessible.

c) Condensations Involving Acyloins or Alkoxy-ketones

Another method of preparing unsaturated fatty acids involves the use of acyloins or alkoxy-ketones. The former may be prepared by Ruzicka's acyloin synthesis[83] and has been extensively used by Baudart,[84] or alternatively, both may be prepared by Bowman's ketone synthesis.[85] In either case the intermediate glycol is readily converted into an unsaturated compound via the dibromide by reaction with zinc dust in acetone in the presence of sodium iodide at 90°C.

The resulting glycol may be separated into two isomeric forms - *threo* and *erythro* which subsequently afford the *trans* and *cis* acids respectively. In general, results are good but in some cases the alkoxy-ketone method is preferable. Ames and Bowman have compared the two methods.[86]

The synthesis of elaidic acid as performed by Baudart is illustrative of the acyloin method[84] and the synthesis of euric acid (13-docosenoic acid) as prepared by Bowman is illustrative of the alkoxy-approach.[87]

(i) Acyloin Method

$$CH_3(CH_2)_7CO_2Et + EtO_2C(CH_2)_7OEt \xrightarrow{Na} CH_3(CH_2)_7COCH(OH)(CH_2)_7OEt \xrightarrow{Ni/H_2}$$

$$CH_3(CH_2)_7CH(OH)CH(OH)(CH_2)_7OEt \xrightarrow[(2)Zn]{(1)HBr/AcOH} CH_3(CH_2)_7CH{=}CH(CH_2)_7Br$$

(Two isomers separated)

$$\xrightarrow{(1)KCN\ (2)\ OH^-} CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$$

(ii) Alkoxy-ketone Method

$$CH_3(CH_2)_7CH(OMe)COCl + HC(CO_2R)_2(CH_2)_{10}CO_2R \xrightarrow{NaOEt} CH_3(CH_2)_7CH(OMe)COC(CO_2R)_2(CH_2)_{10}CO_2R$$

(R=$-CH_2Ph$)

$$\xrightarrow[(2)-CO_2\ (3)Al(OPr^i)_3]{(1)Hydrogenolysis} CH_3(CH_2)_7CH(OMe)CH(OH)(CH_2)_{11}CO_2H \xrightarrow[(2)H^+\ (3)Zn]{(1)HBr/AcOH}$$

(Two isomers separated)

$$\longrightarrow CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO_2H$$

In each case, relatively pure *cis* and *trans* acids were obtained.

d) Chain Extension by Anodic Synthesis

In 1849, Kolbe demonstrated that electrolysis of an aqueous solution of an alkali metal carboxylate, yielded carbon dioxide and a hydrocarbon.[88] Since then, the reaction has been extensively investigated and has been found to constitute a valuable and remarkably simple method for the

synthesis of many organic compounds including fatty acids.[89]

The synthesis of fatty acids is achieved by electrolysis of an alkaline solution of a monocarboxylic acid along with the half ester of a dibasic acid.

$$RCO_2^- + {}^-O_2C(CH_2)_nCO_2Me \longrightarrow R(CH_2)_nCO_2Me + 2CO_2$$

The method may be adapted to the synthesis of unsaturated fatty acids provided that, the double bond is present in the monocarboxylic acid, and is separated from the carboxyl group to be eliminated by at least two carbons. Baker and Gunstone have synthesised *cis*-10-hexadecenoic acid, *cis*-12-octadecenoic acid and *cis*-14-eicosenoic acid in this manner.[90]

Although the procedure is simple, its application is limited as the reaction is accompanied by a number of side reactions. The synthesis of 19-docosynoic acid has been attempted by Klok et al. using a method based on Kolbe's anodic synthesis.[74]

8-Undecynoic acid and tridecanedioic acid mono-methyl ester were used as starting materials and electrolysis afforded a complex mixture of at least 17 components, of which methyl 19-docosynoate was a minor one. Isolation and purification yielded 19-docosynoic acid in 15% yield. It was concluded that although the procedure consisted of few reaction steps it was not suitable for the large scale preparation of long chain unsaturated fatty acids.

$$CH_3CH_2C{\equiv}C(CH_2)_6CO_2H + HO_2C(CH_2)_{11}CO_2Me \xrightarrow[(2)\text{isolation and purification}]{(1)56V,0.6A/50\text{-}60^oC}$$

$CH_3CH_2C{\equiv}C(CH_2)_{17}COOH$
19-Docosynoic acid (19%)

The mechanism and synthetic applications of the Kolbe reaction to the synthesis of fatty acids and other lipids have been thoroughly reviewed.[91,92,93]

e) The Wittig Reaction

The Wittig reaction involves the addition of an alkylidenephosphorane to a carbonyl compound followed by elimination of phosphine oxide from the intermediate betaine to give the alkene.[94]

$$Ph_3P{=}CHR + R'CHO \longrightarrow Ph_3P^{+}CHRC(\bar{O})H_2R' \longrightarrow Ph_3PO + RCH{=}CHR'$$

cis/trans

The reaction proceeds under mild conditions and the position of the double bond is not in doubt. In its original form however, little steric control was possible over the reaction, but subsequent investigations on the mechanism have revealed ways of controlling the selectivity of the reaction. Thus, by suitable adjustment of the reaction conditions and reagents, the *cis*[95,96] or *trans*[97] alkene may be obtained as the predominant product.

The Wittig reaction, or one of its simple modifications, has been used by several workers to synthesise unsaturated fatty acids. A typical example is the synthesis of octadec-9-en-12-yoic acid (crepynoic acid), a naturally occurring plant acid, by Jones and co-workers. This involved the coupling of non-3-yn-1-ol as its triphenylphosphonium bromide with methyl-8-formyloctanoate in the presence of butyllithium. Under these conditions, the reaction proceeds to give the *cis* configuration and methyl crepynate is obtained in 51% yield. Subsequent hydrolysis yields the acid.[98]

$$CH_3(CH_2)_4C{\equiv}CCH_2CH_2OH \xrightarrow{PBr_3} CH_3(CH_2)_4C{\equiv}CCH_2CH_2Br \xrightarrow[\text{reflux}]{PPh_3/MePh}$$

$$CH_3(CH_2)_4C{\equiv}CCH_2CH_2\overset{+}{P}Ph_3\overset{-}{B}r \xrightarrow[(2)OCH(CH_2)_7CO_2Me\ (3)KOH/EtOH]{(1)Bu^nLi/ether} CH_3(CH_2)_4C{\equiv}CCH_2CH{=}CH(CH_2)_7CO_2H$$
cis

It is evident that the synthesis may be readily adapted to the synthesis of monounsaturated fatty acids and Jones et al. have used the method to synthesise a variety of isotopically labelled fatty acids.[99,100]

The limitation of the reaction is that the aldehyde esters are difficult to obtain, methyl 8-formyloctanoate in the above scheme being obtained by ozonolysis of methyl oleate. The stereochemical and mechanistic aspects of the Wittig reaction have been thoroughly reviewed.[101,102]

f) The Preparation and Stereospecific Reduction of Acetylenic Compounds

The preparation of unsaturated acids via acetylenic precursors provides a versatile method which has been widely and successfully exploited. The usefulness of alkynes is based on the fact that firstly, acetylenic compounds are readily alkylated and secondly, the resulting alkenoic acids are essentially *cis* or *trans* depending on whether the reduction is effected catalytically or by chemical means.

Catalytic reduction of acetylenes generally affords high yields of the *cis* alkene. Prior to 1952, palladium[103] and nickel[66] were used extensively. Hydrogenation invariably resulted in a mixture of saturated, *cis* and unreacted acetylenic acids the *cis* acid being isolated by crystallisation. In 1952, Lindlar produced a catalyst (palladium on calcium carbonate partially poisoned with lead acetate) which ensured that hydrogenation ceases after the *cis* alkene is formed and does not proceed

to give the fully saturated acid.[104,105] Employment of this catalyst results in the *cis* alkenoic acids in very high yields.[90]

In contrast to the general mode of catalytic reduction, *trans* acids may be formed by chemical reduction of the acetylenic acid. Methods that have been used include the use of zinc and acetic acid, metals and alkalis, or reduction of the halogen halide mono-addition product. Most of these methods result in a complex mixture because of the formation of by-products, bond migration and isomerisation. In contrast, Campbell and Eby and other workers reported that dialkylacetylenes are smoothly reduced by sodium or lithium in liquid ammonia in the presence of a hydrogen donor to give the *trans* isomer.[106,107]

In 1928, 13-docosynoic (behenolic) acid was obtained by condensing the sodium derivative of 1-decyne with methyl 11-bromododecoate.[108]

$$CH_3(CH_2)_7C{\equiv}CNa + Br(CH_2)_{11}CO_2Me \longrightarrow CH_3(CH_2)_7C{\equiv}C(CH_2)_{11}CO_2H$$

Ten years later, 5-tetradecynoic acid was prepared by the condensation of 1-decyne with 3-chloropropyl toluene-p-sulphonate in the presence of sodamide, the condensation product being subsequently converted to the acid via the nitrile.[109]

$$CH_3(CH_2)_7C{\equiv}CH + C_6H_4MeSO_2(CH_2)_3Cl \longrightarrow CH_3(CH_2)_7C{\equiv}C(CH_2)_3Cl$$

$$\xrightarrow[(2)OH^-]{(1)KCN} CH_3(CH_2)_7C{\equiv}C(CH_2)_3CO_2H$$

Another ten years elapsed before the general usefulness of this reaction was demonstrated by Strong and co-workers who showed that acetylenic compounds interact, as their sodium derivatives or Grignard complexes,

with α,ω-dihalogeno compounds.[110] The resulting halo-acetylenic compound is readily converted into an acid, partial catalytic hydrogenation of which afforded the *cis* alkenoic acid.

$$CH_3(CH_2)_mC{\equiv}CH + I(CH_2)_nCl \longrightarrow CH_3(CH_2)_mC{\equiv}C(CH_2)_nCl \longrightarrow$$

$$CH_3(CH_2)_mC{\equiv}C(CH_2)_nCO_2H \longrightarrow \underset{cis}{CH_3(CH_2)_mCH{=}CH(CH_2)_nCO_2H}$$

The procedure was first described for the synthesis of *cis*-6-undecenoic acid from 1-hexyne and 1-chloro-4-iodobutane.[110] The method was subsequently extended to incorporate the synthesis of *cis* and *trans*-11-octadecenoic (vaccenic) acids (the *trans* acid being obtained by selenium catalysed isomerisation of the *cis* analogue)[66] and a series of short chain acetylenic acids (C_7-C_{14}).[111] Since then the method, or general variations, has been used for the synthesis of a number of unsaturated acids.

Huber has reported the synthesis of several octadecenoic acids.[112] The *cis* acids were first synthesised by partial hydrogenation of the acetylenic acids and the *trans* acids formed by isomerisation using selenium. Ames and co-workers have reported the synthesis of long-chain acetylenic acids by the condensation of a ω-acetylenic acid with an alkyl halide in the presence of sodamide in liquid ammonia.[113,114] In these preparations, the carboxyl group of the ω-acetylenic acid is first protected by reaction with dimethylamine via the acid chloride. This resulted in the formation of an N,N-dimethylamide of a long chain acetylenic acid.

$$HC{\equiv}C(CH_2)_mCO_2H \xrightarrow[(2)Me_2NH]{(1)SOCl_2} HC{\equiv}C(CH_2)_mCONMe_2 \xrightarrow[(2)CH_3(CH_2)_nBr]{(1)NaNH_2/NH_3}$$

$$\longrightarrow CH_3(CH_2)_nC{\equiv}C(CH_2)_nCONMe_2$$

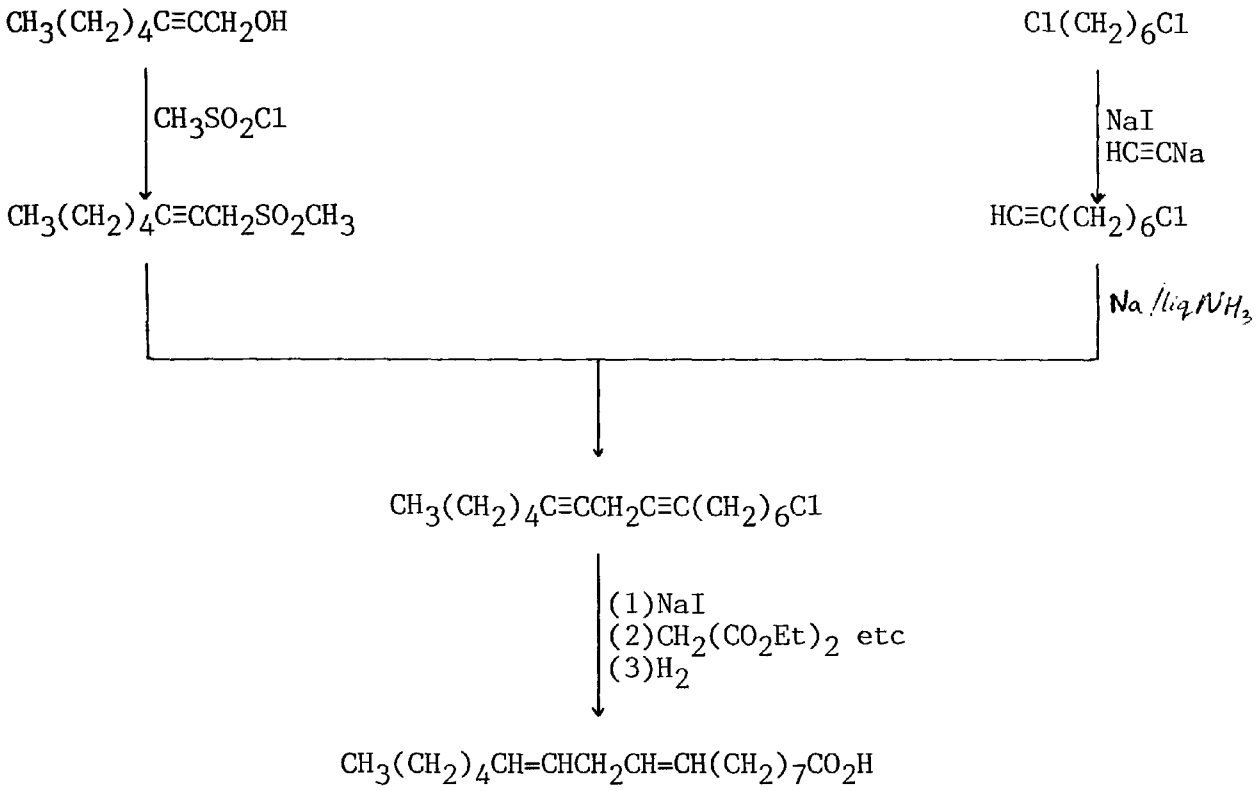
This was converted into the dihydroxy-acid which subsequently afforded the *cis* alkenoic acid in a similar manner to the methods used by Ames and Bowman.[86]

Alternatively, acetylenic acids may be prepared by condensation of ω-bromo-acids with the lithium salt of an alkyne although it is reported that yields are sometimes poor.[115]

$$CH_3(CH_2)_nC{\equiv}CH + Br(CH_3)_mCO_2H \xrightarrow{LiNH_2} CH_3(CH_2)_nC{\equiv}C(CH_2)_mCO_2H$$

More recently, the synthesis and stereospecific reduction of acetylenic compounds has been employed by Gunstone et al. in their syntheses of all the octadecynoic[116] acids and the *cis*[117] and *trans*[116] octadecenoic acids. The *cis* acids were prepared by catalytic hydrogenation of acetylenic acids in the presence of Lindlar's catalyst and the *trans* acids by reduction of acetylenic acids with lithium in liquid ammonia. Gunstone reports that the method in most cases gave the *trans* acid with little or no acetylenic acid and little evidence of double bond migration. The exception was 2-octadecynoic acid which, reduced in this manner, furnished stearic acid and thus the *trans* acid had to be made by an alternative procedure.

Similar reactions have been applied to the synthesis of PUFA. The synthesis of linoleic acid by Raphael and Sondheimer is illustrative.[103]



A similar method is employed by Gensler and Thomas[118] except that the coupling of a propargyl bromide with acetylenic Grignard reagents is catalysed by copper(I) chloride as opposed to using the propargyl methanesulphonate employed by Raphael and Sondheimer.

Subsequent modifications to these methods have led to the synthesis of all the non-conjugated *cis,cis*-octadecadienoic acids[119] and other octadecadienoic[120] acids. Acetylenic precursors as their Grignard complexes and Lindlar's catalyst have also been used in the synthesis of arachidonic acid.[121] The synthesis of PUFA with a 1,4-diene system via acetylenic compounds has been reviewed by Osbond.[122]

The attractiveness of the acetylenic approach to the synthesis of monounsaturated fatty acids is principally threefold. Firstly, it provides a convenient route for the synthesis of a number of acetylenic acids of desired chain lengths and position of unsaturation from common

precursors. Secondly, whereas most of the other methods reviewed above yield a mixture of *cis* and *trans* and, on occasions, positional isomers, the acetylenic acids may be stereospecifically reduced to yield the desired product as the predominant product. Finally, and quite importantly, the starting materials in this scheme - 1-bromoalkanes, acetylene, alkanediols and/or dichloroalkanes - are readily accessible and commercially available in contrast to the starting materials required by some of the other methods reviewed in this Section.

In view of these reasons and considering the range of acids that were to be synthesised, the acetylenic approach was adopted as the backbone of the synthetic programme.

In Part Two, the synthetic aspects of this work are considered from a practical and mechanistic viewpoint. Additionally, the intermediates prepared are fully characterised. Some are novel, others, although prepared previously, are only mentioned in passing in the literature. From this viewpoint, emphasis is placed on the spectroscopic characterisation of these precursors.

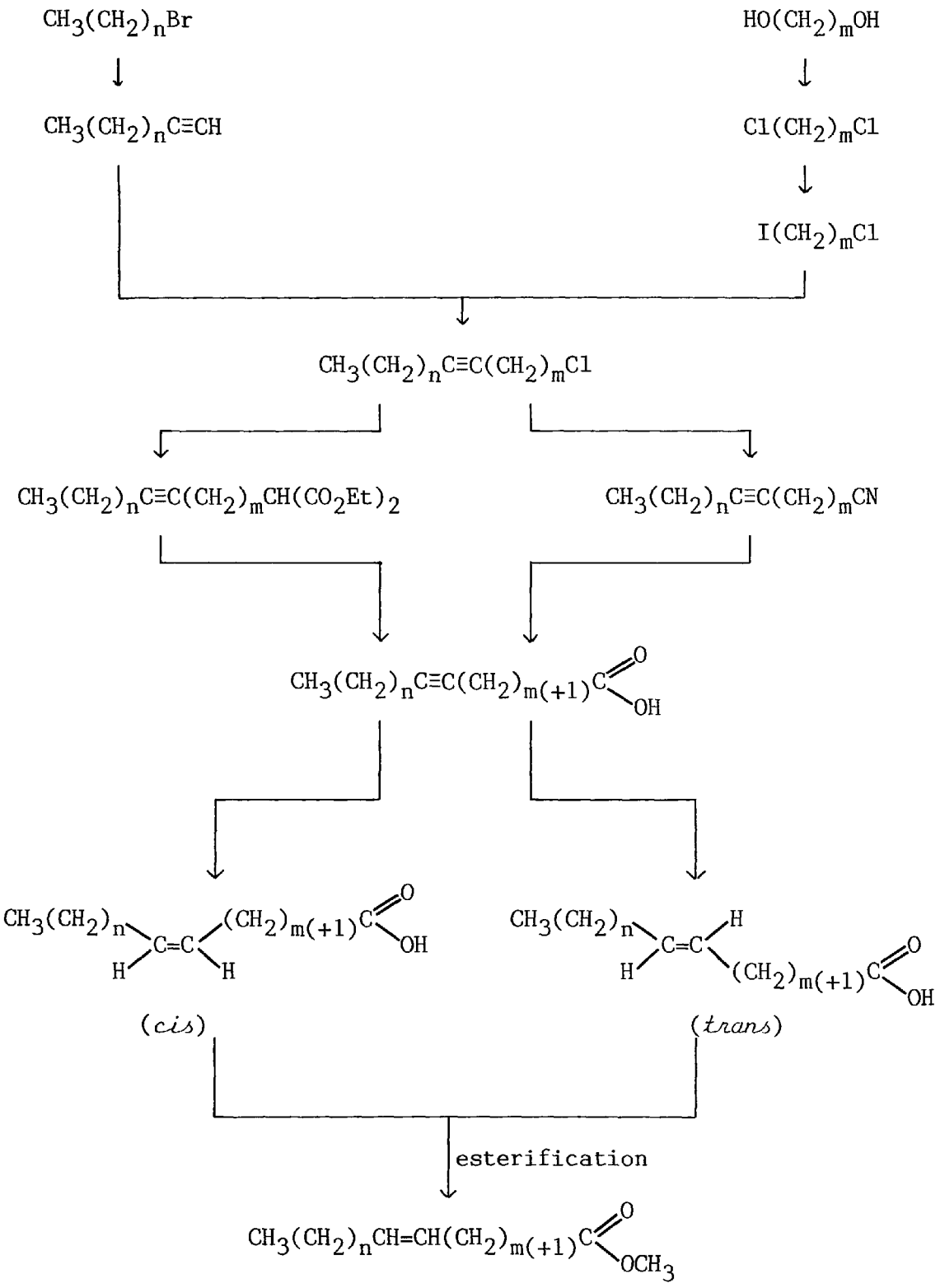
PART TWO

RESULTS AND DISCUSSION

SECTION ONE

THE SYNTHESIS OF INTERMEDIATES USED IN THE SYNTHESIS OF MONOUNSATURATED FATTY ACIDS

A series of monoenoic fatty acids of 12, 14, 16, 18, 20 and 22 carbon chain lengths were synthesised via the general scheme outlined below;



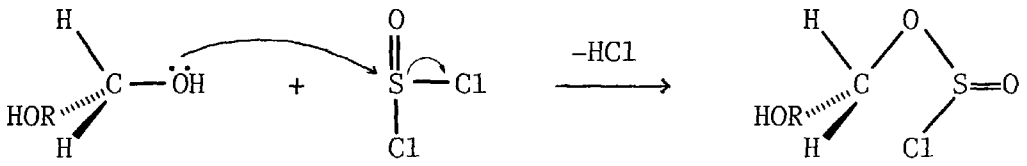
The preparation of the necessary intermediates is now discussed.

1 The Synthesis of α,ω-Dichloroalkanes

α,ω-Dichloroalkanes not commercially available were synthesised in a similar manner to the procedure described by Crombie for the preparation of 1,7-dichloroheptane.[123] It involved the reaction of purified thionyl chloride with a α,ω-alkanediol in the presence of anhydrous pyridine.

$$HO(CH_2)_mOH \xrightarrow[\text{pyridine}]{SOCl_2} Cl(CH_2)_mCl$$

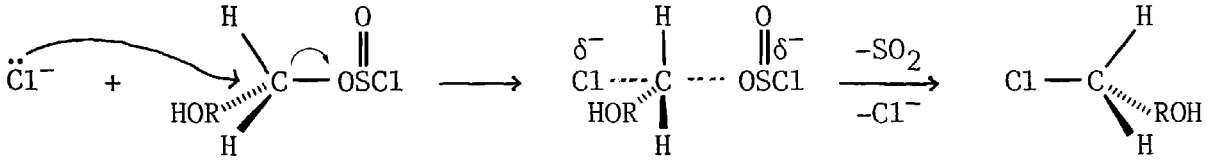
A chlorosulphite ester is first formed with retention of configuration, the CH_2-O bond not being broken during the reaction.



R = $(CH_2)_{m-1}$

In the presence of pyridine, hydrogen chloride liberated in the formation of the chlorosulphite ester is converted by pyridine into $C_5H_5NH^+$ and Cl^-. The chloride ion, being an effective nucleophile, attacks the chlorosulphite ester "from the back" in a normal S_N2 reaction.

$$HCl + C_5H_5N \longrightarrow C_5H_5{}^+NH + Cl^-$$



R = $(CH_2)_{m-1}$

$$Cl(CH_2)_mOH \xrightarrow[(2)Cl^- \text{ etc}]{(1)SOCl_2} Cl(CH_2)_mCl$$

Boiling points and yields of dichloroalkanes are summarised in Table 11. Reaction times increase with increasing chain length and this is accounted for in terms of steric constraints associated with S_N2 reactions. Briefly, the attacking nucleophile must approach the substrate closely to expel the leaving group. The ease of approach depends on the steric nature of the substrate. Sterically bulky substrates, in which the carbon atom is "shielded" from attack by the rest of the molecule, react at a slower rate than substrates in which the carbon is more readily accessible. Thus, increasing chain lengths of alkanediols increases the steric bulk and hence decreases the rate of reaction. Conversion was confirmed from spectroscopic analysis of the reaction products the main features of which are now briefly summarised.

The important IR absorptions attributable to the chloride in dichloroalkanes absorb near 1280 cm^{-1} for CH_2-Cl deformation (in-plane bending) and at 722 and 650 cm^{-1} for C-Cl stretch. In addition, dichloroalkanes exhibit the following absorptions as a result of C-H stretching and bending.

A strong, sharp band between 2940 and 2855 cm^{-1} for CH_2 aliphatic stretch, and a sharp, medium to strong intensity band at 1435-1457 cm^{-1} which may be attributed to CH_2 bending (scissoring). Furthermore, a profusion of absorptions occur throughout the region between 1250 and 770 cm^{-1} because of skeletal vibrations and these gradually weaken as the chain length increases.

A reference to standard IR correlation tables indicates that the strong,

TABLE 11

Reaction Times, Boiling Points and Yields of α,ω-Dichloroalkanes of General Formula $Cl(CH_2)_mCl$

α,ω-Dichloroalkane	m	Reaction Time (hrs)	b.p.(°C)	mm	Yield(g)	Yield(%)[a]
1,4-Dichlorobutane	4	2	35-36	2.5	190.6	96.7
1,5-Dichloropentane	5	-	42-43	2.5	-	-
1,6-Dichlorohexane	6	-	45-46[b]	0.8	-	-
1,8-Dichlorooctane	8	3.5	57-58[c]	0.5	239.6	95.6
1,9-Dichlorononane	9	5	75-76[d]	0.5	235.4	93.4
1,10-Dichlorodecane[e]	10	6	82-83[f]	0.5	233.0	96.1
1,12-Dichlorododecane[g]	12	8	99-100[h]	0.5	218.8	92.5

FOOTNOTES

a) Based on α,ω-Alkanediol

b) Literature value 99°C/28mm[103]; 98-100°C/20mm[118]

c) Literature value 118-119°C/14mm[112]

d) Literature value 86-89°C/2mm[112]

e) m.p., 15.6°C

f) Literature value 115-120°C/4mm[112]; 93°C/0.4mm[116]

g) m.p., 29.0°C

h) Literature value 170-172°C/1mm[124]; 108-112°C/0.3mm[116]

sharp absorption at 720 cm^{-1} may be accounted for by two absorptions. One of these is C-Cl stretching. In addition however, hydrocarbons exhibit a sharp, intense absorption at 720 cm^{-1}. This is attributable to CH_2 asymmetric in-plane rocking which occurs in carbon chains where there are more than four methylene chains in the molecule. This absorption increases in intensity with increasing chain length in direct proportion to the number of methylene groups in the chain. With shorter chain molecules, CH_2 rocking absorptions can lie between 1050 and 720 cm^{-1}. The absorption observed at around 720 cm^{-1} in longer chain dichloroalkanes therefore, is a composite of two vibrations.

The major IR absorbtion frequencies of α,ω-dichloroalkanes recorded as pure (neat) thin films are summarised in Table 12 and are in agreement with literature spectra.[125] The IR spectrum of 1,8-dichlorooctane is illustrated in Figure 4.

^1H NMR spectra of dichloroalkanes, with the exception of 1,4-dichlorobutane and 1,5-dichloropentane, exhibit three signals. These may be assigned to protons on the carbons α to the chloride, the β methylene protons and the polymethylene protons of the hydrocarbon chain. These give rise to a triplet, quintet and a multiplet respectively.

The polymethylene protons in longer chain dichloroalkanes appear as a single broadened absorption peak because of the similar chemical shifts and coupling constants of the polymethylene protons. This is a characteristic feature of all long-chain aliphatic hydrocarbons. Generally, these protons absorb between 1.96 and 1.27 ppm, shifting upfield with increasing chain length.

β Methylene protons absorb between 1.70 and 1.60 ppm as a crude quintet,

TABLE 12
Major IR Absorption Frequencies of α,ω-Dichloroalkanes

Absorption Frequency (cm^{-1})	Assignment
2995-2853	Strong, sharp CH_2 symmetric (2853 cm^{-1}) and asymmetric (2926 cm^{-1}) aliphatic stretching.
1457[a]	Strong, sharp CH_2 bend (scissoring).
1285[b]	Strong, sharp CH_2-Cl deformation (in-plane bending).
1250-770	Profusion of absorptions arising from skeletal vibrations that decrease in intensity with increasing chain length.
722[c]	Strong absorption arising from firstly, CH_2 asymmetric in-plane rocking for carbon chains consisting of four or more methylene groups and secondly, C-Cl stretch.
650	Strong, sharp C-Cl stretch.

FOOTNOTES

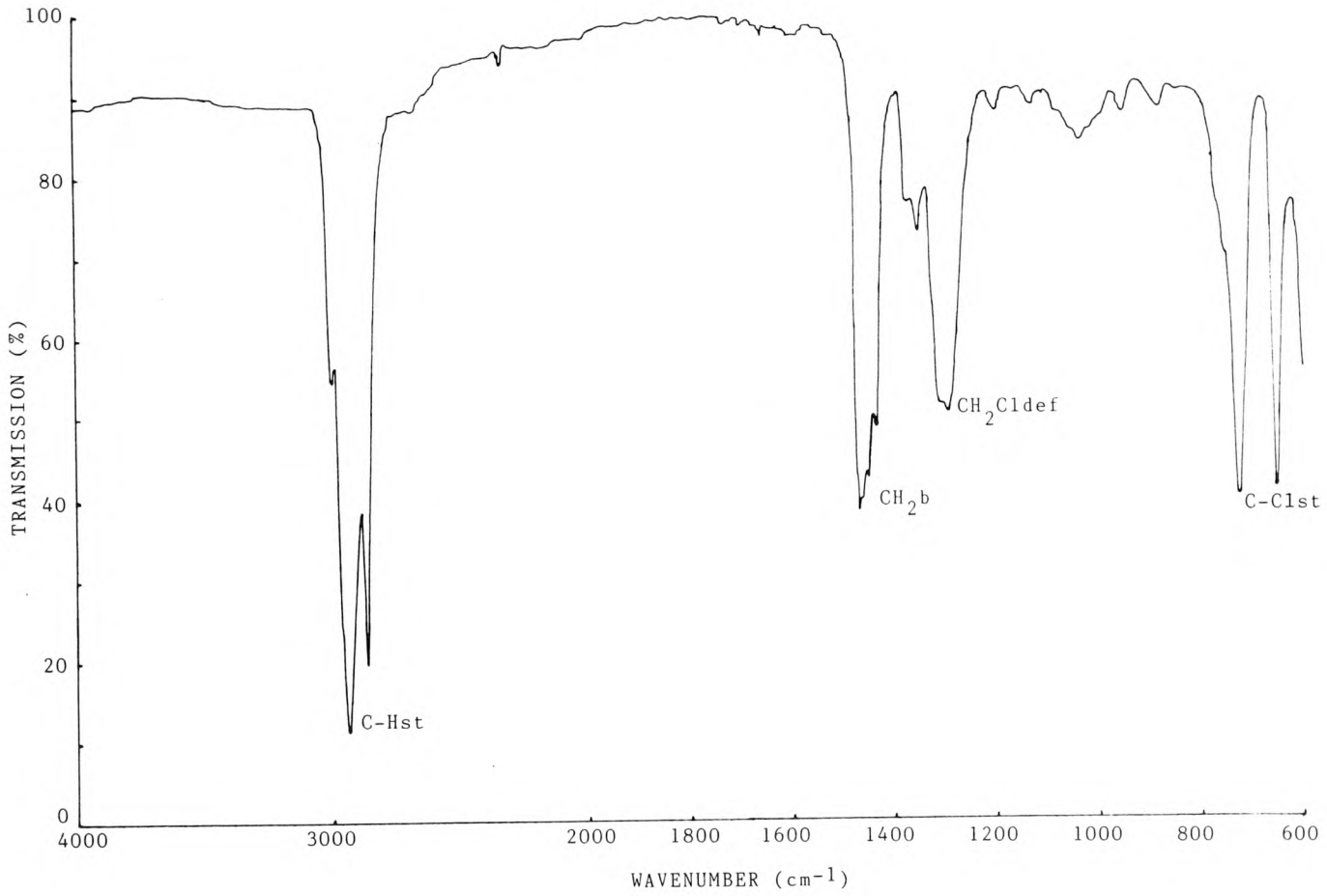
a) 1435 cm^{-1} in 1,4-dichlorobutane and 1445 cm^{-1} in 1,5-dichloropentane.

b) 1293 cm^{-1} in 1,4-dichlorobutane.

c) 740 cm^{-1} in 1,4-dichlorobutane.

FIGURE 4 IR Spectrum of 1,8-Dichlorooctane

$Cl(CH_2)_8Cl$



again shifting upfield with increasing chain length. Virtual (long-range) coupling with protons other than the vicinal ones cause this quintet to distort and broaden.

Protons attached to carbons α to the chloride, absorb between 3.50 and 3.58 ppm and the triplet formed is sharply defined (J=6.84 Hz). The chemical shift of this signal may be explained in terms of deshielding caused by the chloride, which reduces the valence electron density around the protons attached to the α carbon.

^{1}H chemical shifts and assignments of α,ω-dichloroalkanes are summarised in Table 13 and are in agreement with previously recorded data.[126,127] The spectrum of 1,8-dichlorooctane is illustrated in Figure 5.

TABLE 13

^{1}H NMR Chemical Shifts and Assignments of α,ω-Dichloroalkanes

$$\underset{a}{ClCH_2}\underset{b}{CH_2}\underset{c}{(CH_2)_{m-4}}\underset{b}{CH_2}\underset{a}{CH_2Cl}$$

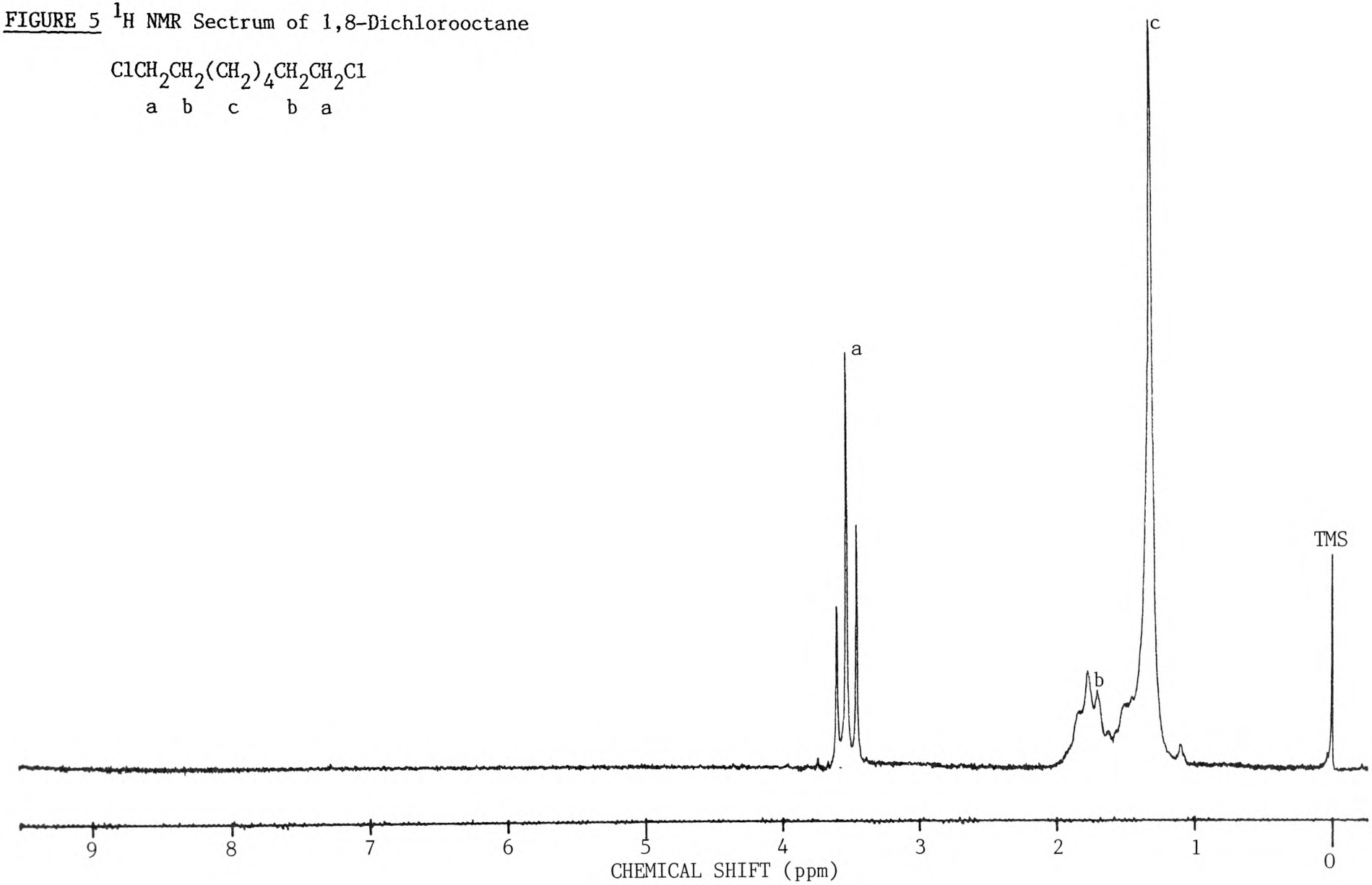
α,ω-Dichloroalkane	m	Shift and Assignment (ppm) a	b	c
1,4-Dichlorobutane	4	3.58	1.96	-
1,5-Dichloropentane	5	3.56	1.75	1.75
1,6-Dichlorohexane	6	3.54	1.78	1.47
1,8-Dichlorooctane	8	3.52	1.70	1.31
1,9-Dichlorononane	9	3.52	1.64	1.32
1,10-Dichlorodecane	10	3.54	1.62	1.30
1,12-Dichlorododecane	12	3.55	1.62	1.29

^{13}C shielding may be basically broken down into a set of three additive contributions namely local diamagnetic shielding, neighbour anisotropic

FIGURE 5 ^{1}H NMR Sectrum of 1,8-Dichlorooctane

$ClCH_2CH_2(CH_2)_4CH_2CH_2Cl$

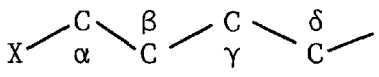
a b c b a



shielding and local paramagnetic shielding.

Very briefly, local diamagnetic shielding describes the isotropic circulation of local electrons around the nucleus. The greater the electron density at the nucleus, the greater the diamagnetic contribution and the further upfield the applied resonance occurs. The neighbour anisotropy effect describes the effect of local electron circulations around the atoms which surround the carbon under observation, and of interatomic electron currents between atoms. Local paramagnetic shielding arises from field induced mixing of the electronic ground state with excited electronic states. It may be visualised as resulting from anisotropic local electron circulations around the particular nucleus.

These contributions to shielding are governed by inter- and intra-molecular electronic effects such as electronegativity, hybridisation and shielding effects which among other factors, all contribute to the shielding of a ^{13}C nucleus, and generally combine in a complex manner. An outstanding feature of ^{13}C chemical shifts is that substituent contributions are additive. This is particularly so for straight-chain aliphatic carbons, where carbon shieldings can be divided up into a number of additive contributions produced by substituents, X, in α, β, γ and δ positions:



The chemical shift of C-1 in dichloroalkanes lies between 44.18 and 45.37 ppm; a downfield shift of approximately 31 ppm compared to the shift in the corresponding alkane. The effect can be understood in part, though not entirely, in terms of the inductive chloride group removing

electron density from the carbon 2p orbitals. The extent of the deshielding experienced depends very much on the electronegativity of the substituent. Generally, the more electronegative the substituent, the greater the chemical shift. Theory predicts[128] this charge transfer to be propagated along the carbon backbone producing alternating effects and falling off with inverse third power of the distance. However, no effect of substituent electronegativity can be found at the β and γ carbons (where the induced shifts are generally consistent regardless of substituent). The implication is therefore that the substituent induced shift of the α carbon, can only be partly because of an inductive effect and that additional effects must be operative. This is evident in dichloroalkanes where, in terms of the inductive effect produced by substituent electronegativity alone, the chemical shift of C_1 (44-45 ppm) is somewhat anomalous. This is attributable to a phenomenon known as the heavy atom effect which is experienced with halogen substituents, in contrast to the general trend exhibited by other substituents.

Generally, substituent effects at the β carbon are found to be fairly constant and independent of the nature of the substituent. A downfield shift of approximately 11 ppm in dichloroalkanes is experienced as a result of the chloride substituent in comparison to the resonance in corresponding hydrocarbons. Hence the shifts for C-2 lie between 29.64 and 32.82 ppm in dichloroalkanes. Attempts to interpret this effect have been made.[129] A conclusive answer is lacking, however, since the effect is obviously a sum of competing contributions.

Carbons γ to a substituent exhibit upfield shifts due primarily to sterically induced polarisation of C-H bonds. According to this concept, a steric perturbation of a C-H bond leads to a drift of charge along the

bond towards the carbon, thus causing orbital expansion and hence increased shielding.[130] Accordingly, the γ carbons of dichloroalkanes undergo an upfield shift of approximately 2-4 ppm with increasing chain length compared to the corresponding hydrocarbon, absorbing between 24.56 and 27.10 ppm.

Substituent effects over four bonds are generally negligible in aliphatic systems (<1 ppm) since in the energetically favoured conformations adopted, there is no nonbonded interaction. Other smaller effects such as electric field effects however, are operative. This is the case with dichloroalkanes, the chemical shifts of the chain carbon atoms falling between 28.84 and 29.62 ppm.

^{13}C chemical shifts and assignments are summarised in Table 14 and are in agreement with previously published data.[131,132] The ^{13}C NMR spectra of 1,9-dichlorononane is illustrated in Figure 6. Also shown in Figure 6 is the ^{13}C NMR spectrum of 1,9-nonanediol. Conversion to 1,9-dichlorononane is confirmed by the absence of the C-OH shift at around 61.2 ppm

2 The Synthesis of α-Chloro-ω-iodoalkanes

α,ω-Dichloroalkanes prepared previously were converted to α-chloro-ω-iodoalkanes by reaction with sodium iodide in dry acetone.

$$Cl(CH_2)_mCl \quad + \quad NaI \quad \longrightarrow \quad Cl(CH_2)_mI$$

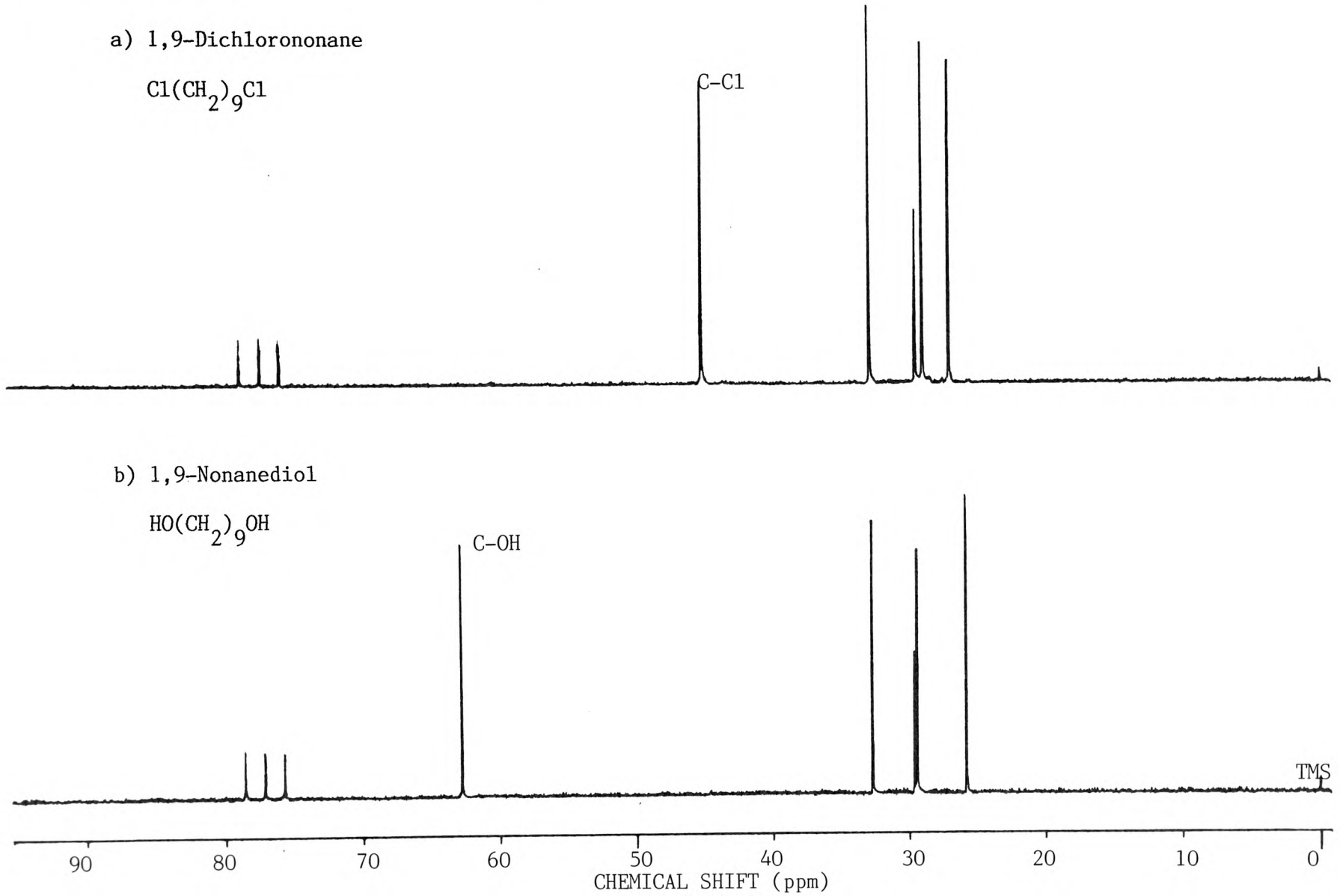
Several variations have been reported for the preparation of chloroiodoalkanes.[103,123] Huber prepared 1-chloro-5-iodopentane in 70% yield by refluxing 7.14 moles of 1,5-dichloropentane with 2.38 moles of sodium iodide in 2 l of acetone for ten hours.[112] Gensler and Thomas

TABLE 14

^{13}C NMR Chemical Shifts and Assignments of α,ω-Dichloroalkanes

α,ω-Dichloroalkane	m	Shift and Assignment (ppm)											
		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
1,4-Dichlorobutane	4	44.26	29.64	29.64	44.26	-	-	-	-	-	-	-	-
1,5-Dichloropentane	5	44.81	32.04	24.56	32.04	44.81	-	-	-	-	-	-	-
1,6-Dichlorohexane	6	45.22	32.82	26.45	26.45	32.82	45.22	-	-	-	-	-	-
1,8-Dichlorooctane	8	45.37	32.82	27.02	29.03	29.03	27.02	32.82	45.37	-	-	-	-
1,9-Dichlorononane	9	45.15	32.79	26.95	28.84	29.34	28.84	26.95	32.79	45.15	-	-	-
1,10-Dichlorodecane	10	45.22	32.82	27.10	29.13	29.62	29.62	29.13	27.10	32.82	45.22	-	-
1,12-Dichlorododecane	12	45.18	32.78	26.95	28.93	29.51	29.51	29.51	29.51	28.93	26.95	32.78	45.18

FIGURE 6 ^{13}C NMR Spectra of 1,9-Dichlorononane and 1,9-Nonanediol



prepared 1-chloro-6-iodohexane in 43% yield by refluxing 1,6-dichlorohexane with an equimolar amount of potassium iodide in 10% aqueous acetone.[118] Ahmad and Strong report the use of a three molar excess of sodium iodide on the assumption that the reaction is reversible.[110] This is considered unnecessary, however, as the employment of a solvent such as acetone, in which sodium iodide is soluble but sodium chloride is not, ensures the reaction results in adequate yields of chloroiodoalkane. The use of an excess of sodium iodide resulted in an increase in the recovery of α,ω-diiodoalkane.

Optimum results were obtained by refluxing dichloroalkane with an equimolar amount of sodium iodide for about 6 hours in a 4:1 solvent:dichloroalkane ratio. Constant stirring is required to avoid violent bumping that occurs as sodium chloride precipitates.

The reaction proceeds via an S_N2 reaction mechanism to give a crude product which is revealed by GLC analysis to be a mixture unreacted dichloroalkane, chloroiodoalkane and diiodoalkane. The chloroiodoalkane was subsequently isolated by fractional distillation under reduced pressure. Boiling points and yields of chloroiodoalkanes are summarised in Table 15.

Yields decreased with increasing chain length and lengthening reaction times made no significant difference to the recovery of chloroiodoalkanes. GLC analysis indicates that on increasing chain length, recovery of unreacted dichloroalkane increased (26.5 to 40%) and recovery of chloroiodoalkane and diiodoalkane decreased.

This trend may be partially explained in terms of the steric nature of the substrate as discussed for the synthesis of dichloroalkanes where,

TABLE 15

Boiling Points and Yields of α-Chloro-ω-iodoalkanes of General Formula $Cl(CH_2)_mI$

α-Chloro-ω-iodoalkane	m	b.p.(°C)	mm	Yield(g)	Yield(%)[a]
1-Chloro-3-iodopropane[b]	3	57-58	10	-	-
1-Chloro-4-iodobutane	4	43-44[c]	2.5	126.4	72.3
1-Chloro-5-iodopentane	5	66-68[d]	2.5	121.6	65.4
1-Chloro-6-iodohexane	6	65-66[e]	0.8	116.0	58.8
1-Chloro-8-iodooctane	8	71-72[f]	0.5	179.2	50.4
1-Chloro-9-iodononane	9	82-83[g]	0.5	104.8	45.4
1-Chloro-10-iododecane	10	90-91[h]	0.5	71.8	29.8
1-Chloro-12-iodododecane	12	108-109[i]	0.5	72.2	27.4

FOOTNOTES

a) Based on α,ω-Dichloroalkane

b) Commercially available (Aldrich) - distilled prior to use

c) Literature value 93-94.5°C/17mm[110]

d) Literature value 125-127°C/36mm[112]

e) Literature value 73-74°C/0.7mm[103]

f) Literature value 101-105°C/2.5mm[112]; 112-116°C

g) Literature value 123-126°C/4mm[116]; 120-123°C/0.48mm[67]

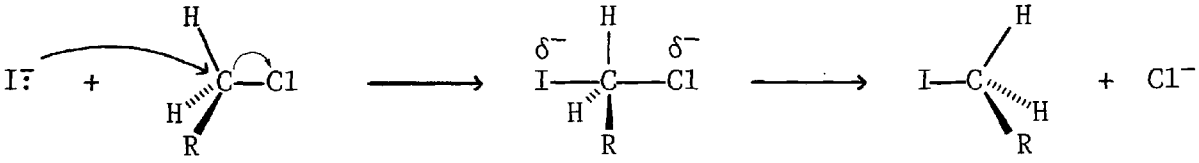
h) Literature value 111-115°C/0.4mm[116]

i) Literature value 105-110°C/0.3mm[116]

increasing chain lengths, decrease the rate of reaction. In addition, however, the nature of the attacking nucleophile and leaving group (in this case, the iodide and chloride respectively) are important factors in determining the rate and final yields of a product in S_N2 reactions, and as such, merit consideration.

The reactivity of a given nucleophile in a specific reaction depends on many factors, such as the nature of the substrate, the solvent and even the conditions under which the reaction is run. Nevertheless, the iodide ion, given its comparatively low electronegativity, large size and hence high polarisability, may be regarded as a particularly good nucleophile. In view of this therefore, the yields of chloroiodoalkanes obtained are surprising when compared to those achieved during the synthesis of dichloroalkanes. The effectiveness of the iodide ion is partially offset, however, by its large size. Consequently, when this is considered with the steric bulk of the substrate upon increasing chain length, the active carbon, at which reaction occurs, becomes increasingly less accessible resulting in reduced yields.

Another important consideration in S_N2 reactions is the nature of the leaving group. In reactions such as this, a transition state exists in which the new I-C bond is partially forming at the same time that the old C-Cl bond is partially breaking.



A redistribution of negative charges occurs in the transition state and polarisability of the attacking nucleophile's and the leaving group's

bonded atom is at a premium in forming the transition state. The leaving group is expelled with a negative charge and generally, the best leaving groups are those that best stabilise the negative charge. In the transition state, the charge is normally distributed over both the attacking and the leaving groups. The greater the extent of charge stabilisation by the leaving group, the more stable the transition state and the more rapid the reaction. Necessarily therefore, the chloride ion is much less effective as a leaving group compared to other leaving groups such as the chlorosulphite ion in the preparation of dichloroalkanes, as it is less able to stabilise the negative charge in the transition state.

To summarise therefore, it is a combination of increasing steric effects with increasing chain length, and the extent of charge stabilisation in the transition state (as a result of the leaving chloride ion) which contribute to the observed yields of chloroiodoalkanes.

The IR spectra of chloroiodoalkanes are similar to those of dichloroalkanes with the ubiquitous C-H symmetric and asymmetric stretching (2853-2995 cm^{-1}), bending (1428-1457 cm^{-1}) and rocking (722 cm^{-1}) absorptions, and CH_2-Cl bending (1282-1300 cm^{-1}) and stretching (722 and 650 cm^{-1}) absorptions. Analogous with dichloroalkanes, there is a profusion of absorptions between 1100 and 770 cm^{-1} due to skeletal vibrations which gradually weaken with increasing chain length. In addition, however, there is a strong, sharp CH_2-I deformation (in-plane) bending vibration which absorbs between 1210 and 1170 cm^{-1}.

Major IR absorptions of chloroiodoalkanes are summarised in Table 16 and the spectrum of 1-chloro-6-iodohexane is illustrated in Figure 7.

The ^1H NMR spectra of chloroiodoalkanes are again similar to those of

TABLE 16
Major IR Absorption Frequencies of α-Chloro-ω-iodoalkanes

Absorption Frequency (cm^{-1})	Assignment
2995-2853	Strong, sharp CH_2 symmetric (2853 cm^{-1} and asymmetric (2926 cm^{-1}) stretch.
1457[a]	Strong, sharp CH_2 bend (scissoring).
1287-1282[b]	Strong, sharp CH_2-Cl deformation (in-plane) bending.
1176-1170[c]	Strong, sharp CH_2-I deformation (in-plane) bending.
1180-770	Profusion of absorptions arising from skeletal vibrations that decrease in intensity with increasing chain length.
722[d]	Strong absorption arising from firstly, CH_2 asymmetric in-plane rocking for carbon chains consisting of four or more methylene groups, and secondly, C-Cl stretch.
650[e]	Strong, sharp C-Cl stretch.

FOOTNOTES

a) 1428 cm^{-1} in 1-chloro-3-iodopropane, 1439 cm^{-1} in 1-chloro-4-iodobutane and 1448 cm^{-1} in 1-chloro-5-iodopentane.

b) 1300-1255 cm^{-1} in 1-chloro-3-iodopropane and 1293 cm^{-1} in 1-chloro-4-iodobutane.

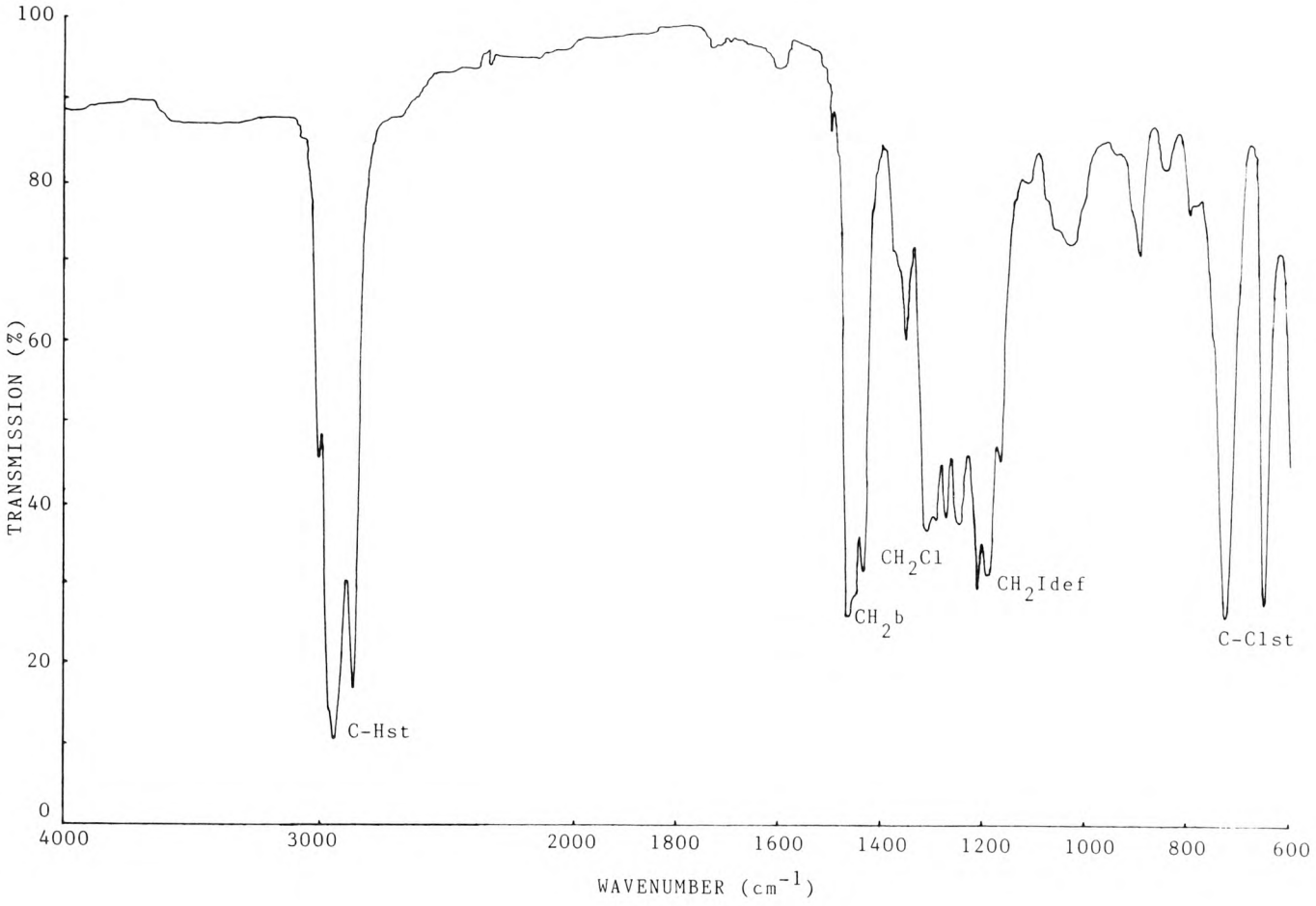
c) 1210-1170 cm^{-1} in 1-chloro-3-iodopropane, 1203 cm^{-1} in 1-chloro-4-iodobutane and 1195 cm^{-1} in 1-chloro-5-iodopentane.

d) Arising from C-Cl stretch only at 757 cm^{-1} in 1-chloro-3-iodopropane.

e) 657 cm^{-1} in 1-chloro-3-iodopropane.

FIGURE 7 IR Spectrum of 1-Chloro-6-iodohexane

$Cl(CH_2)_6I$



dichloroalkanes with an additional signal attributable to protons on the carbon α to the iodide. This absorbs between 3.33 and 3.18 ppm as a well defined triplet (J=7.2 Hz), undergoing a slight downfield shift with increasing chain length. This signal is at a higher field than that for the protons on the α carbon adjoining the chloride (3.64-3.50 ppm), because of the greater local diamagnetic shielding generated by the large iodide ion. The remaining absorptions are roughly identical to those of the dichloro- parent compounds. The methylene protons β to the halogens absorb at 2.22 ppm in 1-chloro-3-iodopropane, undergoing an upfield shift of 0.47 ppm with increasing chain length to 1.75 ppm in 1-chloro-8-iodooctane onwards. The signal is partially superimposed with the main polymethylene proton signal in lower chloroiodoalkanes, but from 1-chloro-8-iodooctane onwards, the signals are separate. The main polymethylene proton absorption also undergoes an upfield shift of about 0.51 ppm with increasing chain length.

^{1}H chemical shifts and assignments are summarised in Table 17 and the spectrum of 1-chloro-6-iodohexane is illustrated in Figure 8.

The substitution of a chloride with iodide has a very marked effect on the ^{13}C NMR spectrum of chloroiodoalkanes. A comparison of ^{13}C chemical shifts for carbons α, β, γ and δ to chloride in chloroiodoalkanes and dichloroalkanes indicates very little variation, absorbing at approximately 45.1, 32.7, 27.1 and 28-29 ppm respectively.

Most notable is the chemical shift of the carbon α to the iodide (5.83-7.24 ppm). The effect of most electronegative substituents upon substituted aliphatic carbon shieldings, are mainly inductive. Generally, the more electronegative the substituent, the greater the inductive

FIGURE 8 ^{1}H NMR Spectrum of 1-Chloro-6-iodohexane

$ClCH_2CH_2(CH_2)_2CH_2CH_2I$
a b c b d

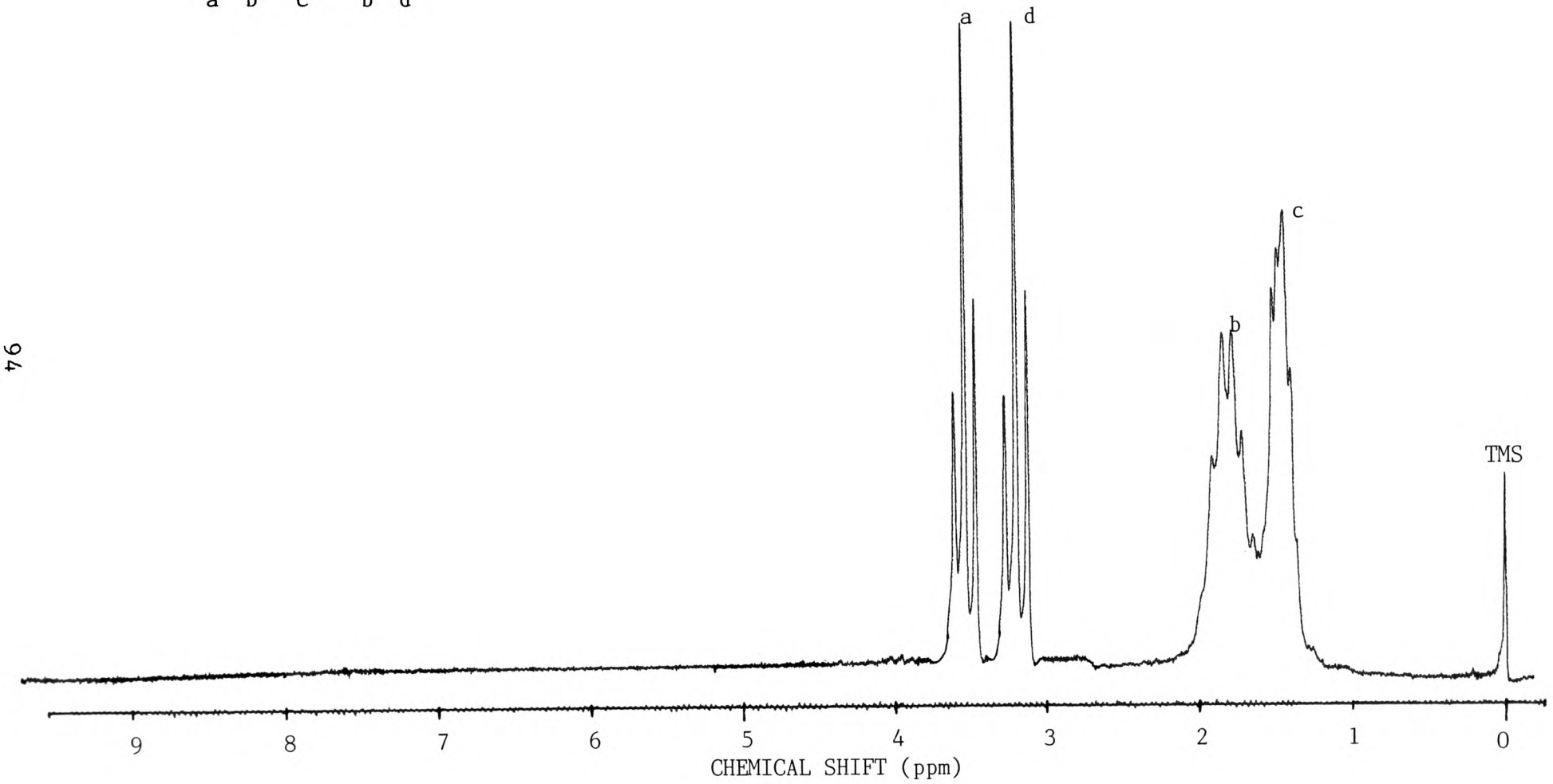


TABLE 17

^{1}H NMR Chemical Shifts and Assignments of α-Chloro-ω-iodoalkanes

$$\underset{a}{ClCH_2}\underset{b}{CH_2}\underset{c}{(CH_2)_{m-4}}\underset{b}{CH_2}\underset{d}{CH_2I}$$

α-Chloro-ω-iodoalkane	m	Shift and Assignment (ppm) a	b	c	d
1-Chloro-3-iodopropane	3	3.64	2.22	-	3.33
1-Chloro-4-iodobutane	4	3.57	1.94	-	3.22
1-Chloro-5-iodopentane	5	3.54	1.79	1.79	3.20
1-Chloro-6-iodohexane	6	3.53	1.81	1.44	3.20
1-Chloro-8-iodooctane	8	3.53	1.75	1.34	3.19
1-Chloro-9-iodononane	9	3.50	1.74	1.31	3.17
1-Chloro-10-iodooctane	10	3.52	1.76	1.30	3.18
1-Chloro-12-iodododecane	12	3.52	1.75	1.28	3.18

effect. This chemical shift therefore is somewhat anomalous. Obviously, electronegative substituent induced shifts do not apply to the heavier halogens, particularly the iodide, and these substituents exhibit an increasing diamagnetic shielding with increasing atomic number. This is produced by the large number of electrons carried by the heavy atoms and phenomenon is known as the heavy atom effect.

The substituent effect of iodide at the β carbon is again found to be relatively constant and independent of the nature of the substituent absorbing at approximately 33.5 ppm, a downfield shift of 0.5-1.0 ppm compared to the shift of a carbon β to a chloride (32.5-32.8 ppm).

The induced shift in the carbon γ to the iodide (30.43-30.61 ppm) is not as large as that in the carbon γ to the chloride (26.96-27.03 ppm) and this again is as a result of the heavy atom effect.

The effect of iodide and chloride substituents on carbons four bonds away is again negligible and the chemical shifts of the carbon skeleton in the higher chloroiodoalkanes resonate between 28.64 and 29.64 ppm.

In the lower chain chloroiodoalkanes (m=3-6), the substituent effects of the chloride and iodide on their respective neighbouring carbons vary from those experienced by the higher chloroiodoalkanes due to the additive influence of both substituents.

^{13}C chemical shifts and assignments of chloroiodoalkanes are summarised in Table 18 and the ^{13}C NMR spectrum of 1-chloro-5-iodopentane is illustrated in Figure 9.

3 The Synthesis of 1-Alkynes

Acetylenic compounds including 1-alkynes have been previously prepared with varying degrees of success by a number of methods which include dehydrohalogenation of dihaloalkanes and monohaloalkenes with potassium hydroxide or sodium amide (sodamide).[133] The synthesis of acetylenic compounds have been reviewed by Jacobs.[134]

More recent methods of preparation include the reaction of alkyl halides with a lithium acetylide-ethylenediamine complex in dimethyl sulphoxide.[135] The synthesis of 1-alkynes has been reported from the reaction of lithium ethenyltrialkylborates (readily prepared from lithium acetylide-ethylenediamine and trialkylboranes) with iodine with complete retention of stereochemistry of the boron-carbon bond. 1-Hexyne for example, has been prepared in this manner in 75% yield. The reaction is of value in situations where groups are often relatively resistant to nucleophilic substitution.[136] More recently, it has been reported that

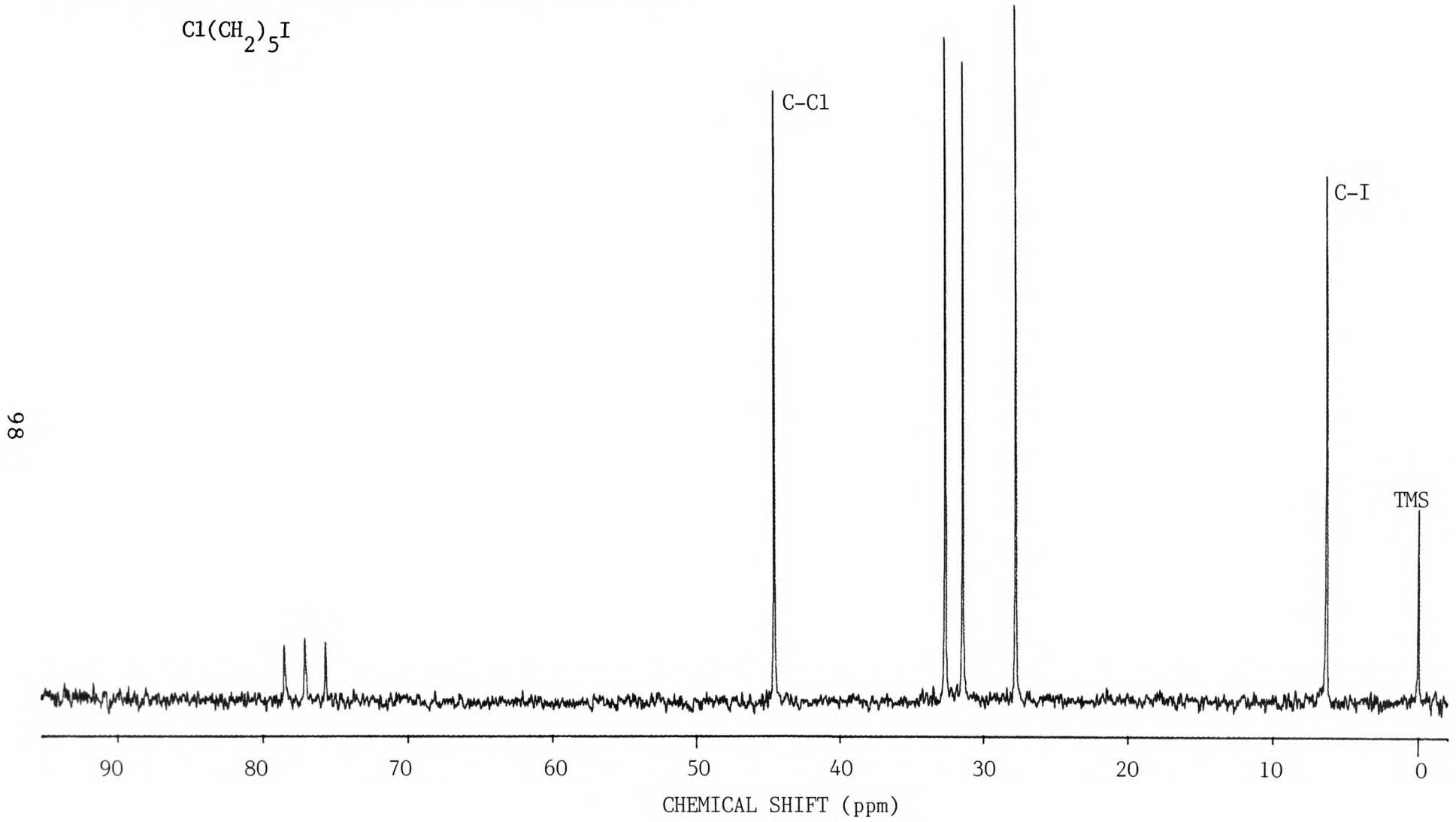
TABLE 18

^{13}C NMR Chemical Shifts and Assignments of α-Chloro-ω-iodoalkanes

α-Chloro-ω-iodoalkane	m	Shift and Assignment (ppm)											
		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
1-Chloro-3-iodopropane	3	42.16	32.82	5.83	-	-	-	-	-	-	-	-	-
1-Chloro-4-iodobutane	4	43.64	30.45	33.26	6.01	-	-	-	-	-	-	-	-
1-Chloro-5-iodopentane	5	44.73	31.62	28.04	32.89	6.40	-	-	-	-	-	-	-
1-Chloro-6-iodohexane	6	44.81	32.33	25.72	29.74	33.31	6.83	-	-	-	-	-	-
1-Chloro-8-iodooctane	8	45.03	32.61	26.96	28.73	28.41	30.43	33.54	7.14	-	-	-	-
1-Chloro-9-iodononane	9	45.11	32.71	26.53	28.81	29.03	29.35	30.36	33.32	7.23	-	-	-
1-Chloro-10-iododecane	10	45.13	32.62	26.10	28.81	29.34	29.34	28.43	30.49	33.56	7.23	-	-
1-Chloro-12-iodododecane	12	45.21	32.80	27.03	28.64	29.64	29.64	29.64	29.64	29.04	30.61	33.61	7.2

FIGURE 9 ^{13}C NMR Spectrum of 1-Chloro-5-iodopentane

$Cl(CH_2)_5I$



potassium 3-amino-propylamide reacts rapidly with vinyl sulphides at room temperature yielding 1-alkynes with a high degree of selectivity via an elimination reaction.[137]

In this investigation, 1-alkynes were synthesised by the reaction of sodium acetylide with 1-bromoalkane in liquid ammonia.

$$CH_3(CH_2)_nBr \quad + \quad NaC{\equiv}CH \quad \longrightarrow \quad CH_3(CH_2)_nC{\equiv}CH$$

Sodium acetylide has previously been prepared by passing acetylene into a solution of sodium in liquid ammonia at the boiling point.[138] A far more rapid and satisfactory method of preparing sodium acetylide consisted of adding sodium to a solution of acetylene in liquid ammonia with stirring at such a rate that the entire body of the reacting solution did not become blue at any time.[139]

Less troublesome and somewhat more adaptable however, is the preparation of sodium acetylide via sodamide in liquid ammonia. This was the method used in this investigation. Sodamide was first prepared in situ by the reaction of sodium with liquid ammonia in the presence of finely divided iron as a catalyst.

$$2Na \quad + \quad 2NH_3 \quad \xrightarrow{Fe} \quad 2NaNH_2 \quad + \quad H_2$$

A solution of sodium acetylide in liquid ammonia was formed by passing an excess of acetylene gas into the suspension of sodamide and addition of 1-bromoalkane to this resulted in the formation of a 1-alkyne via an S_N2 reaction mechanism. Yields and boiling points are summarised in Table 19.

TABLE 19

Boiling Points and Yields of 1-Alkynes of General Formula $CH_3(CH_2)_nC{\equiv}CH$

1-Alkyne	n	b.p.(°C)	mm	Yield(g)	Yield(%)[a]
1-Butyne[b]	1	-	-	-	-
1-Pentyne	2	40-41[c]	760	125.9	95.3
1-Hexyne	3	71-72	760	123.9	91.8
1-Heptyne	4	99-100[d]	760	118.8	88.7
1-Octyne	5	19-20[e]	10	103.6	89.5
1-Nonyne	6	33-34	10	94.4	84.6
1-Decyne	7	57-58[f]	10	114.6	79.3
1-Hendecyne	8	73-74[g]	10	102.2	75.6
1-Dodecyne	9	89-90	10	103.2	71.3

FOOTNOTES

a) Based on 1-Bromoalkane

b) Prepared and used without isolation

c) Literature value 39-40°C/760mm[139]

d) Literature value 97-100°C/760mm[118]

e) Literature value 125-127°C/760mm[116]; 126.3°C/760mm[140]

f) Literature value 172-173°C/760mm[116]

g) Literature value 84-86°/15mm[116]

$$2NaNH_2 + HC{\equiv}CH \xrightarrow{\text{liquid } NH_3} NaC{\equiv}CNa + 2NH_3$$

$$NaC{\equiv}CNa + HC{\equiv}CH \xrightarrow{\text{liquid } NH_3} NaC{\equiv}CH \xrightarrow[\text{liquid } NH_3]{CH_3(CH_2)_nBr} CH_3(CH_2)_nC{\equiv}CH$$

Yields of 1-alkynes generally decreased with increasing chain length the reason for which is twofold. Firstly, steric constraints associated with S_N2 reaction mechanisms (e.g. accessibility of attacking nucleophile to site of reaction with increasing chain length of substrate) and secondly, decreasing reactivity of 1-bromoalkanes (due to their decreasing solubility in liquid ammonia) with increasing chain length. Initially, the synthesis of 1-alkynes by previously published methods was unsuccessful and modification of these methods was necessary.

Traditionally, the preparation of 1-alkynes in this manner has involved the the slow addition of a 1-haloalkane (3-4 hours) to sodium acetylide. The flow of acetylene is then discontinued and the ammonia allowed to evaporate overnight. The mixture is then hydrolysed and worked-up. Analysis of the crude reaction product from these preparations revealed that the mixture contained little or none of the desired product, but did contain significant amounts of starting material and/or by-products. By-products are formed by reactions that occur in competition with the formation of 1-alkynes. The principal by-products formed were identified as alkenes, alkylamines, dialkylethers, dialkylacetylenes and acetylene. Their formation and characterisation have been previously reported.[134,141]

In addition to by-products, reaction mixtures from initial runs contained considerable amounts of the starting material, 1-bromoalkane.

Even after modification and optimisation of reaction conditions, resulting in greatly improved yields, the presence of 1-bromoalkane was the most objectional feature of this method as a source of pure 1-alkynes. This material proved the most difficult of any contaminant to remove from the finished product. Even after careful distillation through an efficient fractionating column, a trace of 1-bromoalkane (1-2%) always remained. Fortunately however, the presence of small amounts of 1-bromoalkane in the final product was of no consequence as it was readily removed in the next stage of the synthesis. As a result, no persistent effort was made to overcome this difficulty.

Since the total exclusion of air during the preparation of sodium acetylide was not attempted, the material always contained some sodium hydroxide due to moisture occurring both in the air and commercial ammonia. It also undoubtedly contained small amounts of sodium oxide due to atmospheric oxygen. This was minimised by protecting all outlets with guard cells packed with glass wool. Evaporating ammonia ensured that the atmosphere above the reacting mixture was relatively free of air. Traces of sodamide may also have been present in some cases since in the absence of excess acetylene, the reaction:

$$NaNH_2 + HC{\equiv}CH \longrightarrow NaC{\equiv}CH + NH_3$$

may be somewhat reversible.

The evaporation of ammonia overnight prior to hydrolysis was found to be detrimental in that firstly, it reduced the yields of 1-alkynes formed, through vapourisation and entrainment of sodium acetylide, and the resulting 1-alkyne (particularly the shorter chain length alkynes). Secondly, rearrangement of 1-alkynes can occur through the production of hot, concentrated sodium hydroxide during hydrolysis. It was beneficial

therefore, as much as possible, to maintain the original volume of liquid ammonia in the reaction vessel and also to have adequate amounts present during hydrolysis. Rearrangement of product was further reduced by the addition of ammonium chloride to decompose any excess sodium acetylide or sodamide present prior to hydrolysis. Yields were optimum when 0.5-1.0 litres of ammonia was present for every mole of sodium acetylide.

Optimum results were achieved when rapid addition of 1-bromoalkane (30-45 minutes) was undertaken, and the flow of acetylene continued at a reduced rate until hydrolysis was effected after no more than 4 hours (and less in the case of the shorter chain length 1-alkynes). During this period, the original volume of ammonia was maintained in the reaction vessel by addition as necessary. Periodic cleaning of the gas inlet is necessary so that the flow of acetylene does not become obstructed by the precipitating sodium bromide. Furthermore, as the longer chain 1-bromoalkanes solidify on contact with liquid ammonia, care must be taken to ensure that the flow of 1-bromoalkane into the reaction mixture does not become obstructed. In this context, the use of a small amount of ether or THF as a co-solvent is beneficial. Large amounts of ether or THF however are, if anything, detrimental.

Yields were improved when the reaction was performed at atmospheric pressure and below $-34^{o}C$. The synthesis has been carried out in an autoclave at higher pressures and temperatures, but such conditions are considered beneficial only if 1-chloroalkanes are used as starting materials.[134] High temperatures and pressures if anything tend to promote the fromation of alkylamines in preference to 1-alkynes. Furthermore, in the preparation of sodium acetylide, acetylene is not

very soluble in boiling liquid ammonia but is markedly so below, and at, atmospheric pressure.

As in previous studies it was found that vigorous stirring was essential to good results. The more nearly homogeneous the reaction mixture, the higher the yield of 1-alkyne obtained. Conversion was confirmed by spectroscopic analysis, details of which follow.

In contrast to the above procedure, because of its physical nature, 1-butyne was prepared and used in the next stage of the synthesis without isolation. For this preparation, commercial sodium acetylide in xylene (Aldrich) was used. Liquid ammonia was added to this slurry and acetylene bubbled through whilst freshly distilled 1-bromoethane was added rapidly (30-45 minutes) in a dropwise manner. The flow of acetylene was then discontinued and the mixture allowed to stand, with stirring, before proceeding to the next stage. Precautions were taken to ensure that the original volume of liquid ammonia was more or less maintained throughout.

The most distinctive and characteristic IR absorptions of monoalkyl-acetylenes are the sharp, medium to strong intensity $\equiv$C-H and -C$\equiv$C- stretching at around 3311 cm^{-1} and 2119 cm^{-1} respectively. Although the position of the latter absorption is constant irrespective of chain length, the absorption frequency of the former decreases slightly with decreasing chain length, absorbing at 3310, 3308 and 3304 cm^{-1} in 1-heptyne, 1-hexyne and 1-pentyne respectively.

The high absorption frequency of this C-H acetylenic stretch compared to the C-H aliphatic stretch is a function mostly of the type of hybridisation that is attributed to the bond. The sp-1s C-H bond present

in acetylenic compounds is stronger than the sp^3-1s bond present in saturated aliphatic compounds, resulting in a larger force constant and hence a greater frequency of vibration.

The strong, sharp absorption at 626 cm^{-1} is attributable to ≡C-H out-of-plane bending which absorbs in this region for monosubstituted acetylenes. In addition the overtone of this absorption is exhibited as a medium intensity band at 1239 cm^{-1} where n=4-9 and 1247 cm^{-1} where n=2-3.

The major IR absorptions of 1-alkynes are summarised in Table 20 and the spectrum of 1-nonyne is illustrated in Figure 10.

The ^1H NMR spectra of the shorter 1-alkynes (n=2-4) exhibit four readily identifiable peaks which may be assigned to the terminal methyl protons, the protons of the polymethylene chain, the propargylic protons (i.e. protons on the carbon α to the acetylenic bond), and the acetylenic proton. In 1-pentyne (Figure 11), these signals give rise to a triplet, sextet, a triplet which is further split into doublets, and triplet at 0.96, 1.58, 2.16, and 1.92 ppm respectively.

The spin-spin splitting patterns observed for the acetylenic and propargylic protons in 1-pentyne (and other 1-alkynes) result from long range coupling made possible through the overlapping π orbitals. Generally the magnitude of the coupling constant (J) depends upon the extent of overlap of the carbon-hydrogen σ bond with the π bond. Coupling constants for such interactions in acetylenic compounds are normally 2-3 Hz. In this investigation, the coupling constant between the acetylenic and propargylic protons in 1-alkynes was measured as 2.63 Hz.

TABLE 20
Major IR Absorption Frequencies of 1-Alkynes

Absorption Frequency (cm^{-1})	Assignment
3311[a]	Strong, sharp ≡C-H stretch.
2966-2854	Strong, sharp C-H aliphatic symmetric and asymmetric stretching.
2119	Medium, sharp C≡C stretch.
1464[b]	Sharp, medium to strong intensity symmetrical in-plane CH_2 bend (scissoring).
1429	Medium, sharp CH_3 asymmetric bend absorbs as "shoulder" on CH_2 bend (scissoring).
1376	Medium, sharp CH_3 symmetric bend.
1239[c]	Sharp, weak to medium intensity ≡C-H out-of-plane bending overtone.
722[d]	Strong sharp absorption arising from CH_2 asymmetric in-plane rocking for carbon chains consisting of four or more methylene groups.
626	Strong, sharp ≡C-H out-of-plane bending.

FOOTNOTES

a) 3304 cm^{-1} in 1-pentyne, 3308 cm^{-1} in 1-hexyne and 3310 cm^{-1} in 1-heptyne.

b) 1455 cm^{-1} in 1-pentyne.

c) 1247 cm^{-1} in 1-pentyne and 1-hexyne.

d) 870 cm^{-1} (?) in 1-pentyne, 740 cm^{-1} in 1-hexyne and 728 cm^{-1} in 1-heptyne.

FIGURE 10 IR Spectrum of 1-Nonyne

$CH_3(CH_2)_6C{\equiv}CH$

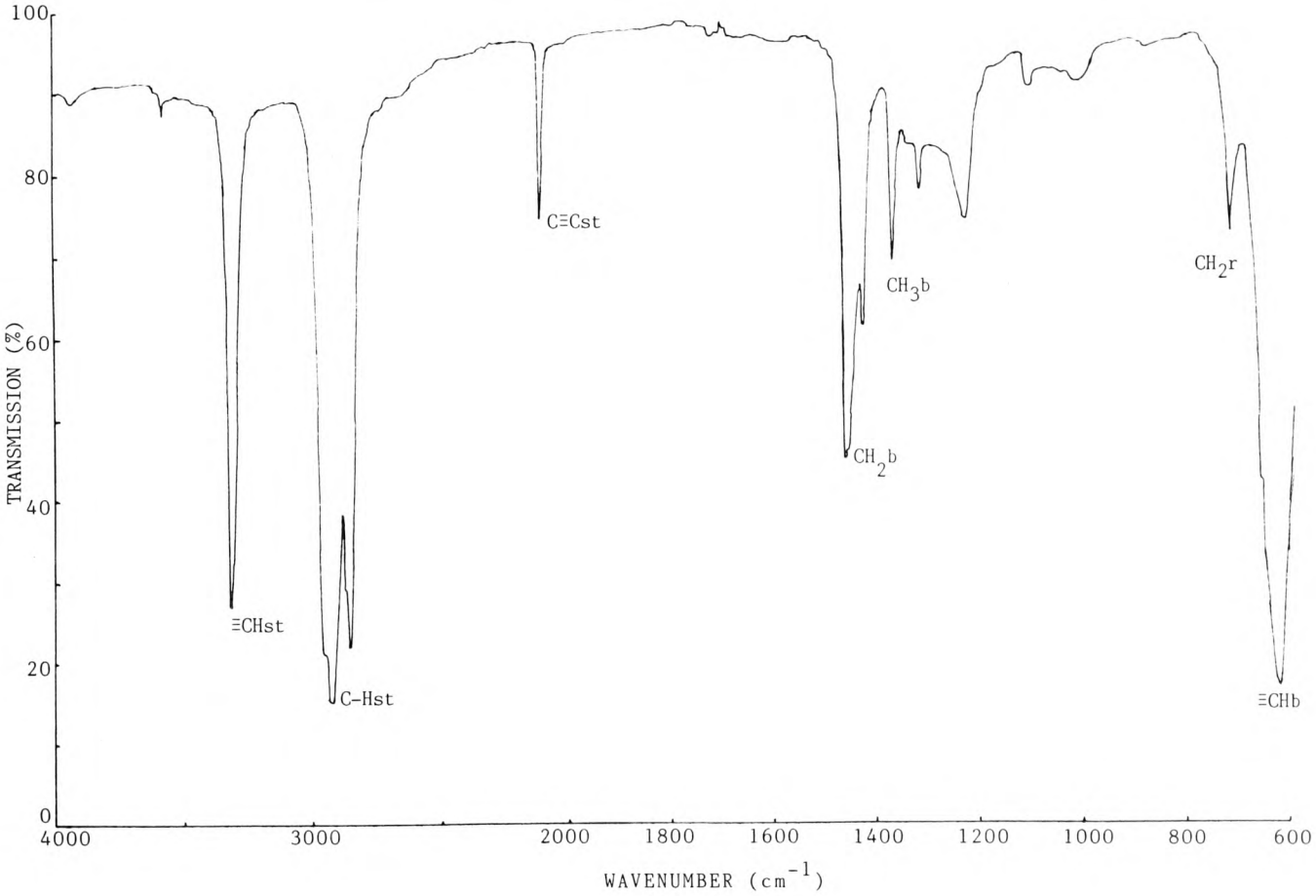
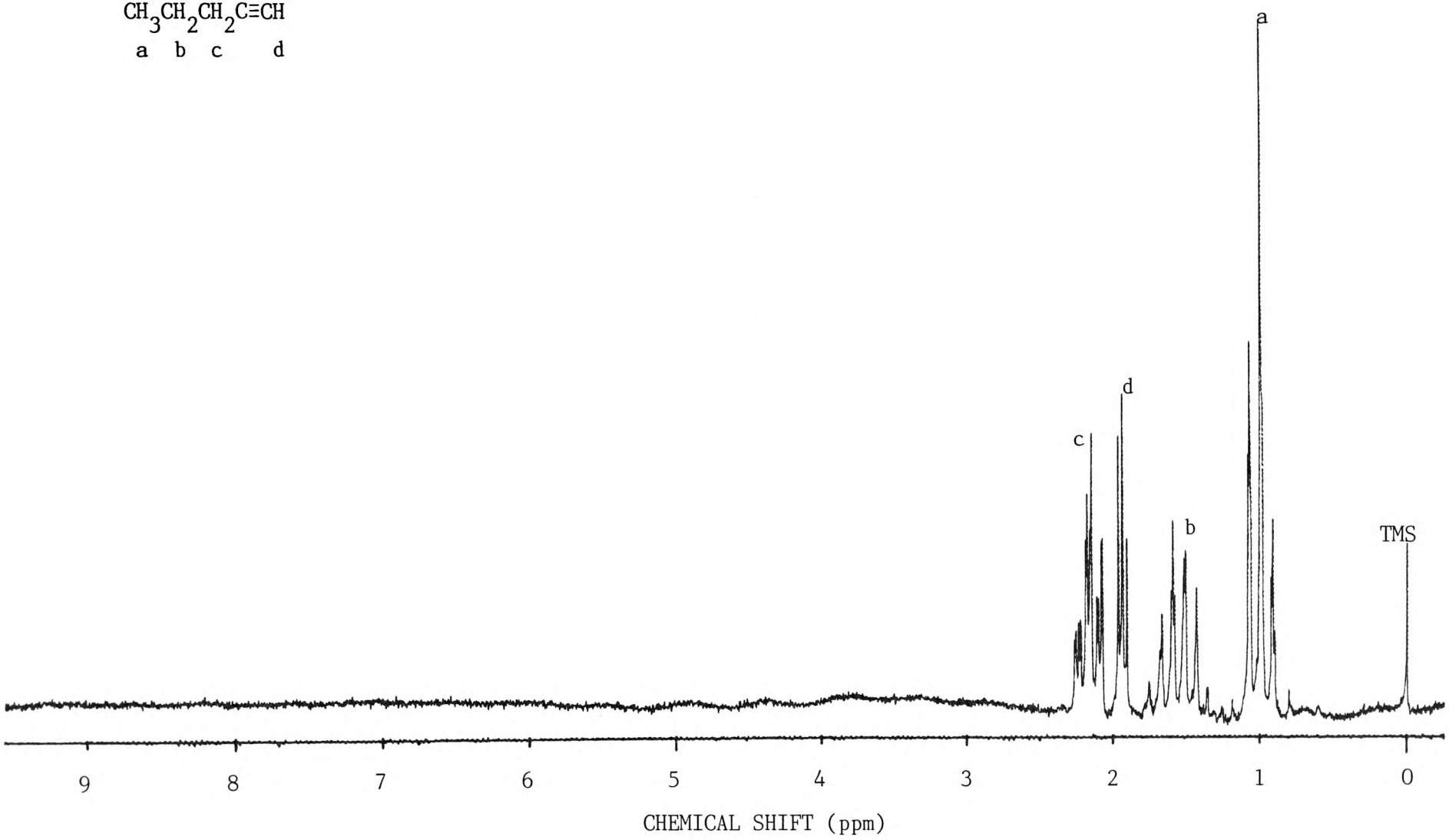
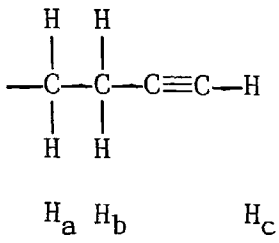


FIGURE 11 ^{1}H NMR Sectrum of 1-Pentyne

$CH_3CH_2CH_2C{\equiv}CH$
a b c d



The propargylic proton absorption in 1-pentyne has a complex splitting pattern which arises from the magnetic non-equivalence of the molecule. Vicinal proton coupling constants for saturated sp^3 hybridised systems are usually 6-8 Hz and were measured here for 1-pentyne as 6.92 Hz. In an acetylenic system such as below therefore:



H_a and H_c are non-equivalent and H_b is thus coupled differently to H_a than to H_c i.e. $J_{ab} \neq J_{bc}$. An analysis of the splitting pattern in 1-pentyne indicates that H_b is first split into a triplet by H_a (J_{ab} = 6.92 Hz) of 1:2:1 intensity and secondly, each of the triplet peaks is split into doublets of equal intensity by H_c (J_{bc} = 2.63 Hz).

As a consequence of this magnetic non-equivalence, each of the protons within a group in 1-pentyne has a slightly different degree of coupling to an adjacent group of protons resulting overall in a slight distortion of signals.

The chemical shifts of the acetylenic and propargylic protons in 1-alkynes are anomalous. Generally, chemical shifts in ^1H spectra may be explained in terms of three main phenomena namely substituent electronegativity, hybridisation (both of which contribute to local diamagnetic shielding), and anisotropy.

On the basis of hybridisation alone, the acetylenic hydrogen by virtue of the sp-1s bond would be expected to have a chemical shift greater than that of an olefinic proton which are attached to sp^2 hybridised

carbons and normally absorb in the range 4.5-7.0 ppm. This is contrary to what is observed (1.90-1.92 ppm). Similarly, propargylic protons absorb between 2.16 and 2.18 ppm, approximately 0.6-0.8 ppm downfield of the shift normally expected for methylene protons on an sp^3 carbon.

Protons whose chemical shifts are not in line with the expected values of any electron-withdrawing or hybridisation effects, and with the presence of an unsaturated system in the vicinity of the protons in question, are said to be influenced by diamagnetic anisotropy, in addition to the applied magnetic field and the usual shielding by the valence electrons around the proton. Alkynes present a striking example of shielding resulting from diamagnetic anisotropy. The anomalously high degree of shielding is considered to arise largely from ring currents in the π electron system. The acetylenic proton lies in a shielded region of the generated anisotropic field. Conversely, the protons on the sp^3 propargylic carbon lie within a deshielded region of the generated anisotropic field, resulting in their anomalously lower shift than normal saturated protons on sp^3 carbons.

The magnitude of the anisotropic field diminishes with distance and, beyond a certain point, there is no effect because of anisotropy. From a comparison of chemical shifts of 1-alkynes summarised in Table 21 however, it is apparent that this effect manifests itself over a distance of at least five carbons.

The methyl proton signal in the longer chain 1-alkynes is unaffected by the triple bond and absorbs constantly at 0.88 ppm. The absorption in shorter chain alkynes exhibits an increasing downfield shift with decreasing chain length (0.88 ppm in 1-octyne to 0.96 ppm in pentyne)

TABLE 21

^{1}H NMR Chemical Shifts and Assignments of 1-Alkynes

$$\underset{a}{CH_3}\underset{b}{(CH_2)_{n-1}}\underset{d}{CH_2}\underset{c}{CH_2}C{\equiv}\underset{e}{CH}$$

1-Alkyne	n	Shift and Assignment (ppm) a	b	c	d[a]	e
1-Pentyne	2	0.96	1.58	2.16	-	1.92
1-Hexyne	3	0.92	1.46	2.17	-	1.92
1-Heptyne	4	0.90	1.43	2.17	-	1.93
1-Octyne	5	0.88	1.29	2.18	1.41	1.90
1-Nonyne	6	0.88	1.28	2.16	1.41	1.92
1-Decyne	7	0.88	1.28	2.17	1.42	1.91
1-Hendecyne	8	0.88	1.28	2.18	1.41	1.90
1-Dodecyne	9	0.88	1.28	2.18	1.42	1.92

FOOTNOTES

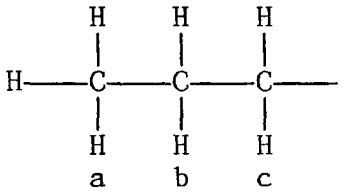
a) Approximate Value.

which may be attributed to the generated anisotropic field. This trend is also exhibited in the ^{1}H spectra of disubstituted acetylenes which are discussed accordingly in subsequent Sections. The signal is thus useful for location of triple bond position along the alkyl chain.

The polymethylene proton absorption of the hydrocarbon chain shifts upfield with increasing chain length from 1.58 ppm in 1-pentyne to 1.28 ppm for 1-nonyne onwards. The well defined sextet exhibited in 1-pentyne increases in complexity with increasing chain length through a multiplet before assuming a single broad absorption peak which is characteristic of long hydrocarbon chains. These two effects result from the methylene

protons exhibiting similar chemical shifts and coupling constants and the greater local diamagnetic shielding of protons uninfluenced by the acetylenic or terminal methyl groups. Consequently therefore, the signal observed represents an "average" chemical shift of the polymethylene protons. In 1-octyne onwards, there is a small absorption at around 1.42 ppm which corresponds principally to protons attached to the carbon β to the acetylenic bond, which are deshielded to a greater extent by the anisotropic field than are the other polymethylene protons.

One other feature is apparent in the 90 MHz ^1H spectra of 1-alkynes with increasing chain length. This is that the spin-spin splitting patterns of principally the methyl and propargylic proton absorption, distort as a result of what is termed virtual coupling. Normally, protons further than one carbon away do not couple. However, in special cases such as long-chain 1-alkynes, protons on alternate carbons appear to interact. They do not couple, but appear to do so. Consider the system:-



$J_{bc} > (\delta_b - \delta_c)$

In such a system, the vicinal protons H_a and H_b clearly couple but H_a appears to couple with H_c as well in spite of the fact that $J_{ac}=0$. This occurs because H_b and H_c have virtually identical chemical shifts and the coupling constant is larger than their chemical shift difference. In such a situation, H_b and H_c behave as a unit and somewhat distort the expected triplet.

Although acetylene has been the subject of several theoretical investigations and the chemical shifts of a number of acetylenic compounds

have been reported and summarised by Emsley,[142] no reference was found for the ^{1}H NMR spectra of linear chain 1-alkynes. Emsley has commented, that as acetylenic compounds are capable of self association, their ^{1}H NMR spectra are markedly solvent and concentration dependent. All spectra in this case were obtained from a 0.4M solution of the 1-alkyne in $CDCl_3$ and results are reproducible within the limits of experimental error to $\pm$0.005 ppm. The ^{1}H chemical shifts of 1-alkynes are summarised in Table 21.

^{13}C NMR chemical shifts and assignments for 1-alkynes are summarised in Table 22. The assignment of the acetylenic carbon signals is relatively simple as the shifts of hydrocarbons in ^{13}C NMR spectroscopy fall within three broad ranges depending on the state of hybridisation of the carbon. The general trend $sp^3 > sp > sp^2$ parallels the order found in ^{1}H NMR. However, whereas the observed shift sequence for ^{1}H is a consequence of neighbour anisotropy, the dominant factor in ^{13}C NMR is the state of hybridisation of the carbon with the transition energy and multiple-bond contributions combining to increase the paramagnetic term. sp-Hybridised carbon shifts generally lie between 65 and 88 ppm and for 1-alkynes in this investigation, were recorded for C-1 and C-2 at 68.08-68.39 ppm and 84.53-84.89 ppm respectively. The higher field of C-1 may partly be explained by the greater degree of shielding as a result of the anisotropic shift generated by the triple bond but, as anisotropic effects in carbon are relatively small, it is more probably due to a reduction in local paramagnetic shielding.

The chemical shift of the propargylic carbon is strongly influenced by the acetylenic bond and when uninfluenced by any other functional group, the resonance tends towards 18.50 ppm. While this shielding effect may

TABLE 22

^{13}C NMR Chemical Shifts and Assignments of 1-Alkynes

1-Alkyne	n	Shift and Assignment (ppm)											
		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
1-Pentyne	2	68.39	84.69	20.61	22.20	13.54	-	-	-	-	-	-	-
1-Hexyne	3	68.25	84.89	18.32	30.83	22.08	13.76	-	-	-	-	-	-
1-Heptyne	4	68.11	84.67	18.45	28.33	31.06	22.28	13.96	-	-	-	-	-
1-Octyne	5	68.08	84.70	18.47	28.52	28.76	31.41	22.62	14.06	-	-	-	-
1-Nonyne	6	68.08	84.67	18.47	28.62	28.87	28.87	31.82	22.72	14.10	-	-	-
1-Decyne	7	68.06	84.70	18.47	28.62	28.87	29.16	29.26	31.92	22.72	14.10	-	-
1-Undecyne	8	68.11	84.55	18.52	28.70	28.94	29.45	29.64	29.31	32.06	22.82	14.12	-
1-Dodecyne	9	68.11	84.53	18.52	28.70	28.94	29.50	29.70	29.75	29.31	32.06	22.82	14.10

be attributable to the diamagnetic anisotropy of the triple bond, the extent of the shift may not be entirely explained in these terms and contributions made by the diamagnetic and paramagnetic shielding terms have to be taken into consideration.[143] There is an increase in electron density at the propargylic carbons which tends to expand the 2p orbitals and consequently increases the local diamagnetic shielding.

In 1-pentyne, the influences of the acetylenic bond and terminal methyl groups act in an additive manner on the propargylic carbon. Consequently, the shift of this signal (20.61 ppm) is deshielded by approximately 2.1 ppm relative to its position in an isolated system (e.g. 1-dodecyne - 18.52 ppm). In contrast, the corresponding signal in 1-heptyne experiences a slight upfield shift (0.2 ppm), due to an increase in diamagnetic shielding, absorbing at 18.32 ppm.

The influence of the acetylenic bond extends at least 6 or 7 carbons and can have quite a pronounced effect on easily assigned signals at the methyl end of the molecule in particularly the shorter chain length 1-alkynes. It is evident from Table 22 that the four carbons at the methyl end of the molecule (with the exception of C-2 in 1-pentyne) undergo an increasing upfield shift the greater the proximity to the acetylenic bond.

The chemical shifts of the remaining sp^3 hybridised carbons of the hydrocarbon chain in longer chain 1-alkynes remain relatively unaffected by the substituents and absorb as expected for such carbons, around 29.69 ppm.

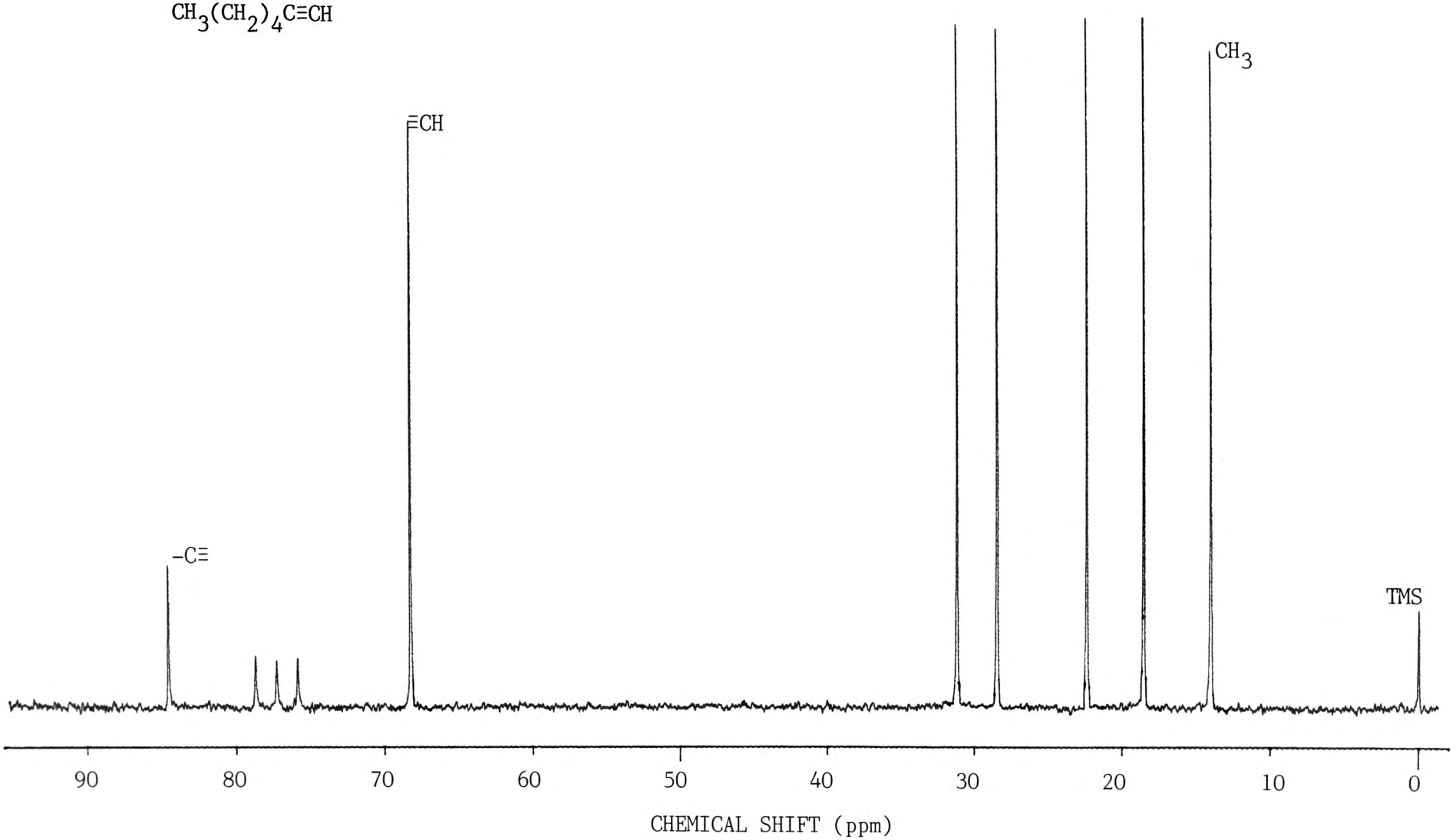
^{13}C NMR spectra have been reported for a number of linear alkynes although in most cases, only the chemical shifts of the sp-hybridised

carbons were reported. Such data that have been reported have been summarised by Dorman et al..[143] Rang,[144,145] Dorman[143] and their co-workers have between them reported the chemical shifts of several 1-alkynes. Chemical shifts in both previous studies were reported relative to CS_2 and once corrected for relativeness to TMS using the expression $192.8-\delta_{CS_2}=\delta_{TMS}$, it is obvious that there is some difference between the ranges of chemical shifts recorded here and those published previously.

Such large disparities in the literature data may be accounted for in terms of solvent and concentration effects. The shifts recorded by Rang et al. were derived from neat solutions, while solutions of the alkyne in 1,4-dioxane (concentration unspecified) and subsequently referenced to external CS_2 standard were used by Dorman et al.. As acetylenes are capable of self association, at such high concentrations as those used by Rang, intermolecular interactions are considerable and undoubtedly influence the chemical shifts. In this investigation spectra were run as 0.4 M solutions of 1-alkyne in $CDCl_3$ referenced to TMS. Within the limits of experimental error, the chemical shifts are reproducible ($\pm$0.05 ppm). The ^{13}C NMR spectrum of 1-heptyne is illustrated in Figure 12.

FIGURE 12 ^{13}C NMR Sectrum of 1-Heptyne

$CH_3(CH_2)_4C{\equiv}CH$



4 The Synthesis of Isomeric 1-Chloroalkynes

A series of isomeric 1-chloroalkynes of chain lengths 10-19 were synthesised by either one of two methods. Initially, these compounds were prepared by the condensation of a 1-alkyne as its sodium salt with a α-chloro-ω-iodoalkane in liquid ammonia.

$$CH_3(CH_2)_nC{\equiv}CH \xrightarrow[(2)I(CH_2)_mCl]{(1)NaNH_2/Liq\ NH_3} CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$$

Due to difficulties encountered during the preparation of some isomers using this method, the synthesis of other 1-chloroalkynes was undertaken by condensing 1-alkyne as its lithium salt with a α-chloro-ω-iodoalkane in an ethereal solvent.

$$CH_3(CH_2)_nC{\equiv}CH \xrightarrow[(2)I(CH_2)_mCl]{(1)RLi/ether} CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$$

In both cases the reaction proceeds via an S_N2 mechanism, the acetylide ion condensing with the α-chloro-ω-iodoalkane with elimination of usually, but not exclusively, iodide. Iodide is preferred to chloride as a leaving group because of its comparatively lower electronegativity, larger size, higher polarisability and hence greater charge stabilisation in the transition state. This is not always the case however, and on occasions, the iodo- derivative was also recovered where chloride had been eliminated in preference to iodide. Their formation was of no consequence however as the 1-iodoalkyne may be used with equal success in the next stage of the synthesis.

4.1 The Synthesis of 1-chloroalkynes via Sodamide in Liquid Ammonia

This method of preparation involved the condensation of a 1-alkyne as its sodium salt with a α-chloro-ω-iodoalkane in liquid ammonia.

$$CH_3(CH_2)_nC{\equiv}CH \xrightarrow[(2)I(CH_2)_mCl]{(1)NaNH_2/Liq\ NH_3} CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$$

The alkynylsodium salt was prepared by the addition of a 1-alkyne to sodamide. α-Chloro-ω-iodoalkane was then added, and the mixture hydrolysed and worked-up to leave a mixture (according to the conditions employed) of product, and/or by-products and/or starting materials.

In contrast to this general procedure, in reactions involving 1-butyne, which was prepared and used without isolation, sodium metal was added to the suspension of 1-butyne in liquid ammonia at such a rate that the mixture did not at any time turn entirely blue. After the addition was complete, the reaction was completed in the usual manner.

Initially, difficulty in preparing 1-chloroalkynes by published procedures[66,90,111,112] were encountered. These difficulties were somewhat analogous to those encountered during the preparation of 1-alkynes in terms of recovery of starting materials and by-products. Even after optimisation, the reaction in some cases did not prove entirely satisfactory. In particular, during the formation of the longer chain length 1-chloroalkynes and 1-chloroalkynes where the position of unsaturation was not central, the product was not recovered without quantities of starting materials and/or by-products.

Spectroscopic analysis of crude products from initial runs, in addition to signals characteristic of starting materials, indicated the presence

of two major classes of by-products namely primary alkenes and alkylamines. Their formation derived from competing substitution and elimination reactions of α-chloro-ω-iodoalkanes with liquid ammonia. As there are two reactive sites at which reactions can occur, a variety of by-products are formed, depending on whether the chloride, iodide or both, are displaced or eliminated. Generally, these by-products are readily removed by distillation or elution on silica. Their main spectroscopic characteristics are summarised below.

By-product	Infrared (cm^{-1})	^1H NMR (ppm)	^{13}C NMR (ppm)
Alkenes	=C-H(st), 3005 C=C(st), 1637 CH=CH_2(bend), 992,907	-CH=, 6.2 =CH_2, 5.0	-CH=, 138 =CH_2, 114 -CH_2-CH=, 27
Alkylamines			
Primary	N-H(st), 3335 (strong, broad doublet) N-H(def), 1651 (strong, sharp) N-H(bend), 910-770 C-N(st) 1065 (sharp, weak)	NH_2, 1.2(singlet) α-CH_2, 2.7(triplet)	C-N, 41-42
Secondary	N-H(st), 3335 (weak, broad) N-H(bend), 910-770 C-N(st), 1150 (sharp, medium)		C-N, 48-49
Tertiary	C-N(st) 1235 (sharp)		C-N, 51-52

Large quantities of the ω-chloro-1-alkene were recovered suggesting that the alkenes are principally formed by dehydrohalogenation at the iodide of α-chloro-ω-iodoalkanes. Their recovery was surprising as although secondary and tertiary alkyl halides readily undergo dehydrohalogenation in the presence of a strong base, the reaction is not normally associated with primary straight chain alkyl halides.

Conditions under which a substantial amount of alkenes were recovered involved the use of an excess of sodamide leading to a situation where the alkynylsodium once formed, competes with the sodamide for the

haloalkane in an S_N2 vs. E2 type situation.

Elimination is favoured with respect to substitution at higher temperatures. Thus, the performance of the reaction at lower temperatures (-55°C) was, whenever possible, beneficial in reducing alkene formation. Alkene formation was further minimised by using only a slight excess of sodamide (sufficient only to destroy any residual 1-bromoalkane present as a contaminant in 1-alkyne). After optimisation, and with appropriate precautions, alkene formation was negligible.

A mixture of primary, secondary and tertiary alkylamines are formed by the S_N2 reaction of alkyl halides with liquid ammonia in direct competition with the formation of 1-chloroalkynes. Primary and secondary alkylamines are the predominant product with trace amounts of tertiary alkylamines.

$$NH_3 + R\text{-}X \longrightarrow RNH_3^+X^- \xrightarrow{OH^-} RNH_2$$

$$RNH_2 + R\text{-}X \longrightarrow R_2NH_2^+X^- \xrightarrow{OH^-} R_2NH$$

$$R_2NH + R\text{-}X \longrightarrow R_3NH^+X^- \xrightarrow{OH^-} R_3N$$

The formation of alkylamines was reduced when the reaction was performed at lower temperatures (-55°C). In cases involving longer chain length starting materials, due to problems of solubility, the reaction was performed at higher temperatures and the formation of some alkylamines in these cases was, for the most part, unavoidable.

In some cases, small amounts of dialkylethers (65 ppm) and alcohols (62 ppm) were also detected. Precautions taken to prevent their formation are the same as those discussed for the preparation of 1-alkynes.

In addition to the performance of the reaction, whenever possible, at lower temperatures, and the use of only a slight excess of sodamide, the overall yields of 1-chloroalkynes were improved by the rapid addition of α-chloro-ω-iodoalkane (30-60 minutes). As in the preparation of 1-alkynes, because longer chain length compounds solidify on contact with liquid ammonia, precautions were taken to ensure that the passage into the reaction mixture did not become obstructed. Again, a small amount of ether or THF was beneficial in this context.

Depending on the chain length of the resulting 1-chloroalkyne, yields were optimum when hydrolysis was effected 2-4.5 hours after the addition of the α-chloro-ω-iodoalkane. For synthesis of the shorter chain length compounds, a delay of 2-2.5 hours sufficed. With synthesis of the longer chain length compounds however, a delay greater than 4 hours was not beneficial. Hydrolysis before this resulted in the increased recovery of starting materials. A long delay prior to hydrolysis resulted in decreased yields of product, because of loss through vapourisation and entrainment, and decomposition.

As in the preparation of 1-alkynes, vigorous stirring was essential and best results were obtained when 0.5-1.0 litres of ammonia was present per mole of 1-alkyne. Furthermore, the presence of adequate amounts of ammonia in the reaction vessel during hydrolysis, was beneficial in preventing rearrangement of the 1-chloroalkyne. Hydrolysis was carefully controlled and was typically effected in a dropwise manner, with stirring, over 1.5-2 hours with iced water and a cooled reaction vessel.

Generally, yields of 1-chloroalkyne and degree of contamination varied with position of unsaturation and the chain length of the resulting

1-chloroalkyne. The C_{10}-C_{12} 1-chloroalkynes were relatively free of contamination and sufficiently pure to proceed to the next stage without purification. The degree of contamination increased with increasing chain length and varying the reaction time made no significant improvement to the yields. 1-Chloroalkynes were purified by either distillation under reduced pressure or elution with petroleum ether/ether on silica. In some cases distillation of the lower boiling point material to leave the product as a residue sufficed.

Where the position of unsaturation in the resulting 1-chloroalkyne was central, yields were generally good but decreased as the position of unsaturation moved towards the chloride. Generally, yields were low for any reactions involving 1-decyne, 1-hendecyne, 1-dodecyne, 1-chloro-10-iododecane and 1-chloro-12-iododododecane and the recovery of these compounds from the reaction mixture was high. In addition, no product was recovered from any reaction involving the use of 1-chloro-3-iodo-propane and reactions involving 1-chloro-4-iodobutane resulted in poor to moderate yields. Addition of co-solvents as reported by Gunstone and co-workers[116,117] made no difference to these two general aspects of the synthesis.

It would appear therefore that the general reaction:

$$CH_3(CH_2)_nC{\equiv}CH \quad + \quad I(CH_2)_mCl \longrightarrow CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$$

via sodamide in liquid ammonia occurs readily only if n is 6 or less. The condensation proceeded satisfactorily when m was between 5 and 9, moderately when m was 4 or 10, poorly when m was 12 and failed completely when m was 3. It may be concluded therefore that this method of synthesis offers a convenient and practical route to 1-chloroalkynes of general formula $CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$ where n is 6 or less and m may

be varied from 4 to at least 10.

The failure of the reaction with the longer chain compounds may be explained partly in terms of the decreasing solubility and consequently decreasing reactivity of these compounds in liquid ammonia. It was felt that with milder reaction conditions that the general scheme may be extended to include the synthesis of compounds not readily prepared by sodamide in liquid ammonia. With this in mind, the feasibility of using the organometallic reagent methyllithium was investigated.

4.2 The Synthesis of 1-Chloroalkynes via Methyllithium

Ziegler's discovery[146] that alkyl halides will react with lithium metal in ether or benzene to yield organolithium compounds has made these reagents readily available and the great synthetic utility of the metallation reaction has been demonstrated and reviewed by a number of workers.[147,148] Until recently, the most commonly used metallating agents were butyl- and phenyllithium. While these are not the most reactive organolithium compounds, they were the most readily available. Today however, with the commercial availability of methyl- and ethyllithium, the trend has been towards using these compounds as the reactivity increases with decreasing molecular weight.

The metallation of acetylenic compounds by organolithium reagents was first attempted in 1965 when it was demonstrated that terminal acetylenic compounds could be polymetallated using butyllithium.[149,150] Phenyllithium has also been used to prepare the lithium derivative of 1-hexadecyne which was condensed with ethylene oxide in the presence of dioxan and liquid ammonia.[117] The resulting octadec-3-yn-1-ol (90% based on 1-hexadecyne) was subsequently converted to cis-3-octadecenoic acid.

More recently, the alkylation of some acetylenic compounds using organolithium compounds has been reported[151] and Gilman and Holland have utilised organolithium compounds to synthesise a number of acetylenic acids.[152] With these exceptions however, the metallation of terminal acetylenes by organolithium compounds has been little exploited.

The synthesis of 1-chloroalkynes via the alkynyllithium salt is not unknown however. Lumb and Smith have reported the synthesis of 1-chloro-4-hexadecyne in this manner from the condensation of 1-tridecyne and 1-chloro-3-iodopropane.[153] Although the preparation of tridecynyllithium and its condensation with 1-chloro-3-iodopropane failed in liquid ammonia, the lithium salt was obtained in dioxan from the reaction between lithium metal and di(tridecynyl)mercury. When this solution of the lithium salt was condensed with 1-chloro-3-iodopropane, a 67% yield of crude 1-chloro-4-hexadecyne resulted.

$$[CH_3(CH_2)_{10}C{\equiv}C]_2Hg \xrightarrow{2Li} 2CH_3(CH_2)_{10}C{\equiv}C^-Li^+$$

$$\xrightarrow{2Cl(CH_2)_3I} \underset{67\%}{CH_3(CH_2)_{10}C{\equiv}C(CH_2)_3Cl}$$

The basic protocol for the preparation of 1-chloroalkynes required the formation of the alkynyllithium salt to which was added the α-chloro-ω-iodoalkane and the reaction mixture subsequently worked-up to leave the product.

The alkynyllithium salts of 1-alkynes were thus first prepared by the dropwise addition of a 1-alkyne with stirring, to a solution of an equimolar amount of methyllithium in diethyl ether, at room temperature.

The reaction proceeded rapidly with the evolution of methane to leave a white precipitate of the alkynyllithium salt.

$$CH_3(CH_2)_nC{\equiv}CH \xrightarrow{MeLi} CH_3(CH_2)_nC{\equiv}C^-Li^+$$

Methyllithium was used as it is the most reactive of the commercially available organolithium reagents. Resulting from the extremely sensitive nature of methyllithium, it must at all times be handled, and the reaction performed, in an oxygen free atmosphere.[154] Furthermore, the success of the reaction is dependent upon all the usual precautionary conditions employed in organometallic reactions[154,155] principally the exclusion of moisture from all reaction apparatus and reagents. Careful solvent and reagent preparation is thus essential.

Initial attempts at the preparation of 1-chloroalkynes, which involved the addition of α-chloro-ω-iodoalkane to the alkynyllithium salt in ether at room temperature, and allowing to stand with stirring, proved unsuccessful. Varying reaction times and conditions (refluxing) resulted in some recovery of 1-chloroalkynes (1-5%) and analysis of the crude "product" from these runs indicated the presence of mostly unreacted 1-alkyne and α-chloro-ω-iodoalkane.

This was surprising as although the carbanionic species formed from 1-alkynes are less powerful nucleophiles than, for example, alkyl Grignard reagents, they nevertheless undergo the usual range of reactions with electrophiles. As there was no doubt as to the formation alkynyllithium salt, and in principal there was no reason why this would not condense with chloroiodoalkane to form the 1-chloroalkyne, the problem was thought to lie in the choice of solvent. Alkynyllithium salts are poorly soluble in ether and precipitate out of solution upon

formation. An alternative reaction medium to ether is thus desirable.

Traditionally, ethereal solvents and hydrocarbons such as pentane and hexane are the most commonly used solvents for organolithium reactions.[156] Although the simple formula "RLi" is usually adequate for representing organolithium compounds in equations, it is doubtful whether such a species can exist except under highly unusual conditions. Under the conditions normally encountered, most organolithium compounds are associated and, in the presence of electron donors, form coordination complexes. It has been reported that organometallic reactions generally proceed more rapidly in ethereal solvents than hydrocarbons.[157] This is because of the much greater solvating properties of ethers than hydrocarbons, as a result of a lower degree of association between the metal and the ether, than between the metal and the hydrocarbon. Furthermore, complexing tends to loosen or ionise the carbon-lithium bond leading to an overall lower energy requirement in the transition state. Consequently therefore, the use of hydrocarbons as solvents were not considered suitable (methyllithium is actually insoluble in hydrocarbons) and tetrahydrofuran (THF) and dioxan were considered as possible alternatives. Such solvents have occasionally been used in the past[158] and it has been reported that complexes formed in such solvents are solvated dimers of higher reactivity than the polymeric forms that exist in less polar solvents.[157]

Generally, the method employed was as follows. The 1-alkyne was added in a dropwise manner to methyllithium in ether at room temperature. Most of the ether was then removed and replaced with either THF or dioxan which had been dried and purified prior to use.[159] α-Chloro-ω-iodoalkane was then added in a dropwise manner and the mixture subsequently hydrolysed

and worked-up. Within this general protocol, in addition to solvent type, the following parameters were varied in order to optimise the yield; reaction time, molar ratios of reactants, and solvent concentration. Furthermore, the reaction was performed at room temperature and under reflux.

The reaction was moderately successful using THF as a solvent with refluxing for about 24 hours (20-30 % recovery) although best results were obtained by refluxing for 24 hours in dioxan with continuous stirring with the presence of a slight excess of the alkynyllithium salt. Yields varied from 38-65% depending on the position of unsaturation. 1-Chloroalkynes not readily prepared via sodamide in liquid ammonia were synthesised with improved yields using this method of preparation. In particular, the synthesis of compounds involving the use of 1-chloro-3-iodopropane and longer chain α-chloro-ω-iodoalkanes, which failed completely with the former method, were prepared using methyllithium.

Furthermore, in addition to the greater solubility of the higher compounds in ethereal solutions, this mode of reaction has a distinct advantage over that of sodamide/liquid ammonia due to the milder reaction conditions. This is, although as in the original reaction, recovery of starting materials is at times quite appreciable, the formation of by-products is eliminated as there are no competing elimination or substitution reactions. However, as with the formation of 1-chloroalkynes from sodamide/liquid ammonia, the iodide was not exclusively eliminated and on occasions, a mixture of 1-chloroalkyne and 1-iodoalkyne was obtained although the 1-chloroalkyne was the predominant product.

It should be pointed out that Lumb and Smith, using a similar method during their preparation of 1-chloro-4-hexadecyne, isolated not only 1-chloro-4-hexadecyne and 1-iodo-4-hexadecyne but also a small amount of solid material which they determined to be nonacosa-12,17-diyne.[153] The formation of this compound probably results from the reaction of 1-tridecynyllithium with both the chloride and iodide of 1-chloro-3-iodopropane.

$$2CH_3(CH_2)_{10}C{\equiv}CLi + Cl(CH_2)_3I \longrightarrow CH_3(CH_2)_{10}C{\equiv}C(CH_2)_3C{\equiv}C(CH_2)_{10}CH_3$$

No evidence of such compounds was however found during this investigation.

Although 1-chloroalkynes synthesised by this method were produced in adequate amounts to proceed to the next stage of the synthesis, employed conditions are not optimised and it is probable that yields could with further investigation be improved.

As stated earlier, the problem is the formation of complexes between the metal and the solvent. The degree of association is lowered by coordination with electron donors and by certain structural features, notably steric hindrance and the capacity to delocalise negative charge. The lower the degree of association, the greater the reactivity. The influence of the solvent is thus very important. In general, electron donating solvents increase the reactivity by lowering the degree of association and increase the carbanionic reactivity by promoting the formation of the lithium cation. Thus, reactivity would be expected to increase in the order ether<THF<dioxan and this was found to be the case.

It seems reasonable that the use of ethereal type solvents such as dimethyl ethylene glycol (diglyme)[158] or crown ether compounds[162,163] would be beneficial. The use of solvents such as hexamethylphosphoramide (HMPA) have been reported in similar reactions by Gilman and Holland in their synthesis of acetylenic acids.[152] Furthermore, it has been reported that some reactions[147,148] involving organolithium reagents benefit in terms of increased reaction rate and improved yields from the presence of bidentate ligands. Examples of these are 1,4-diazabicyclo(2,2,2)octane (DABCO)[161] or tetramethylethylenediamine (TMEDA).[160] Such compounds serve to lower the degree of association and as such, may have a role in this reaction.

Yields and boiling points (where recorded) of 1-chloroalkynes, together with their mode of synthesis, are summarised in Table 23. Additionally, yields and boiling points of the 1-iodoalkynes which were occasionally recovered are also summarised. The preparation of 1-chloro-13-hexadecyne failed completely. This preparation involved condensing 1-butyne with 1-chloro-12-iodododecane in liquid ammonia and the failure of the reaction was most probably because of the poor solubility and hence low reactivity of 1-chloro-12-iodododecane in liquid ammonia. The formation of 1-chloro-13-hexadecyne by this method albeit in poor yield, has however been reported in the literature.[116]

The IR spectra of the majority of 1-chloroalkynes exhibit no distinguishing features that set them apart from any other aliphatic halogenated hydrocarbons (Table 24).

Bands attributable to C≡C stretching which would normally be expected to absorb around 2250 cm^{-1} for disubstituted acetylenes, do not give rise

TABLE 23

Mode of Synthesis, Boiling Points and Yields of 1-Chloroalkynes of General Formula $CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl$

1-Chloroalkyne	m	n	Method[a]	b.p(°C)	mm	Lit. value(°C/mm)	Reference	Yield(g)	Yield(%)[b]
1-Chloro-7-decyne	6	1	Na	69-72	1.0	-	-	20.2	57.3[c]
1-Chloro-4-hendecyne	3	5	Li	69-71	0.5	-	-	11.2	46.4
1-Chloro-5-hendecyne	4	4	Na	66-67	0.5	90-92/1.5	111	4.0	15.3
			Li					12.1	50.6
1-Chloro-6-hendecyne	5	3	Na	63-64	0.5	77-79/1.0	111	25.0	89.7
1-Chloro-7-hendecyne	6	2	Na	64-65	0.8	-	-	21.0	94.3
1-Chloro-7-dodecyne	6	3	Na	75-76	0.5	75-78/0.35	152	20.2	88.6
1-Chloro-4-tridecyne	3	7	Na	94-96	0.6	-	-	0.0	0.0
			Li					11.6	42.4
1-Chloro-5-tridecyne	4	6	Na	90-91	0.8	-	-	7.2	23.3
			Li					17.1	53.7
1-Chloro-6-tridecyne	5	5	Na	88-89	0.4	112-113/2.0	111	34.3	89.4
1-Chloro-7-tridecyne	6	4	Na	87-88	0.5	d	90	28.4	94.7
1-Chloro-9-tridecyne	8	2	Na	e	-	-	-	25.3	82.3
1-Chloro-10-tridecyne	9	1	Na	e	-	-	-	19.2	80.0[c]
1-Chloro-11-tetradecyne	10	2	Na	101-104	0.8	-	-	3.0	8.6
			Li					12.5	50.6
1-Chloro-4-pentadecyne	3	9	Na	111-112	0.6	-	-	0.0	0.0
			Li					10.3	52.3
1-Chloro-5-pentadecyne	4	8	Na	108-109	0.5	-	-	3.8	14.5
			Li					8.7	56.3
1-Chloro-6-pentadecyne	5	7	Na	107-109	0.5	-	-	8.2	37.6
1-Chloro-7-pentadecyne	6	6	Na	120-122	1.0	-	-	11.1	45.9
1-Chloro-9-pentadecyne	8	4	Na	108-109	0.5	-	-	26.0	55.0
1-Chloro-10-pentadecyne	9	3	Na	109-110	0.6	-	-	27.8	77.2
1-Chloro-11-pentadecyne	10	2	Na	112-114	0.8	-	-	4.3	13.3
			Li					18.7	67.2
1-Chloro-7-hexadecyne	6	7	Na	130-131	0.5	205-206/32.0	116	20.6	58.3
1-Chloro-11-hexadecyne	10	3	Na	109-110	0.1	127-126/0.6	116	17.4	55.3
1-Chloro-13-hexadecyne	12	1	Na	-	-	-	116	0.0	0.0
1-Chloro-6-heptadecyne	5	8	Li	124-125	0.5	119/0.3;136-140/2.0	112;116	17.8	44.6
1-Chloro-7-heptadecyne	6	7	Li	119-121	0.5	118-120/0.5;151/2.0	112;116	25.6	84.3
1-Chloro-9-heptadecyne	8	6	Na	119-121	0.5	149-151/2.0	112	28.1	94.0
1-Chloro-11-heptadecyne	10	4	Li	122-124	0.6	159-162/3.5;173/4.5	112,116	13.8	48.5
1-Chloro-13-heptadecyne	12	2	Li	e	-	-	-	9.0	37.2
1-Chloro-7-octadecyne	6	9	Li	e	-	-	-	6.5	30.1
1-Chloro-11-octadecyne	10	5	Li	e	-	-	-	11.3	38.9
1-Chloro-13-octadecyne	12	3	Na	f	-	-	-	2.0	8.6
			Li					7.6	34.4
1-Chloro-9-nonadecyne	8	8	Li	f	-	-	-	7.9	37.3
1-Chloro-10-nonadecyne	9	7	Na	117-118	0.1	-	-	10.0	35.6
1-Iodo-10-nonadecyne				f					
1-Chloro-11-nonadecyne	10	6	Li	122-123	0.2	-	-	9.9	35.4
1-Iodo-11-nonadecyne				157-158	0.2				
1-Chloro-13-nonadecyne	12	4		f	-	-	-	6.8	28.6

FOOTNOTES

a) Na - Sodamide/Liquid ammonia; Li - Methyllithium/dioxan.

b) Based on 1-alkyne unless indicated otherwise.

c) Based on chloroiodoalkane.

d) Used without purificatiion.

e) Compounds isolated by elution with pet. ether/ether on silica.

f) Recovered as residue from distillate.

TABLE 24
Major IR Absorption Frequencies and Assignments of 1-Chloroalkynes (and some 1-Iodoalknes)

Absorption Frequency (cm^{-1})	Assignment
2966-2854	Strong, sharp C-H aliphatic symmetric and asymmetric stretching.
1464	Sharp, medium to strong intensity symmetrical in-plane CH_2 bend (scissoring).
1429	Medium, sharp CH_3 asymmetric bend. Absorbs as "shoulder" on CH_2 scissoring.
1376	Medium, sharp CH_3 symmetric bend. Absorbs as "shoulder" on CH_2-Cl deformation.
1330-1280	Medium, sharp CH_2-Cl deformation (in-plane bending) of the 1-chloro-alkynes.
1186-1176	Medium, sharp CH_2-I deformation (in-plane bending) of the 1-iodoalkynes.
720	Strong, sharp absorption because of C-Cl stretch and CH_2 asymmetric in-plane rocking for carbon chains consisting of four or more methylene groups.
650	Strong, sharp C-Cl stretch.

to any significant IR absorptions in 1-chloroalkynes. This absorption is absent when unsaturation is central, and is of low intensity and difficult to observe even when substitution is asymmetrical. This is because disubstituted acetylenes such as these 1-chloroalkynes, as a result of the local symmetry around the triple bond, are effectively (as is acetylene), IR inactive. The IR spectrum of 1-chloro-9-pentadecyne is illustrated in Figure 13.

The ^{1}H NMR spectrum of 1-chloro-10-pentadecyne (Figure 14) exhibits five absorptions which may be assigned to the terminal methyl protons (0.92 ppm), the polymethylene protons (1.37 ppm), the propargylic protons (2.10 ppm), protons on the carbon β to the chloride (1.68 ppm) and the protons on the carbon α to the chloride (3.52 ppm). Whereas the terminal methyl group and propargylic proton absorptions are distorted, the absorption of the α carbon protons is a well defined triplet (J=6.5 Hz). The propargylic proton absorption is slightly upfield of the corresponding signal in 1-alkynes although again, anomalously high as a result of the deshielding caused by diamagnetic anisotropy.

As a result of long range deshielding induced by the functional groups, the chemical shifts of these absorptions may be altered by the position of unsaturation along the alkyl chain. In particular, a significant downfield shift is induced in the terminal methyl absorption as the triple bond approaches this position. Using the nomenclature (n-x) where n is the chain length and x is the number of carbon atoms from the triple bond to the terminal carbon, the following generalisation may be drawn:

FIGURE 13 IR Spectrum of 1-Chloro-9-pentadecyne

$CH_3(CH_2)_4C{\equiv}C(CH_2)_8Cl$

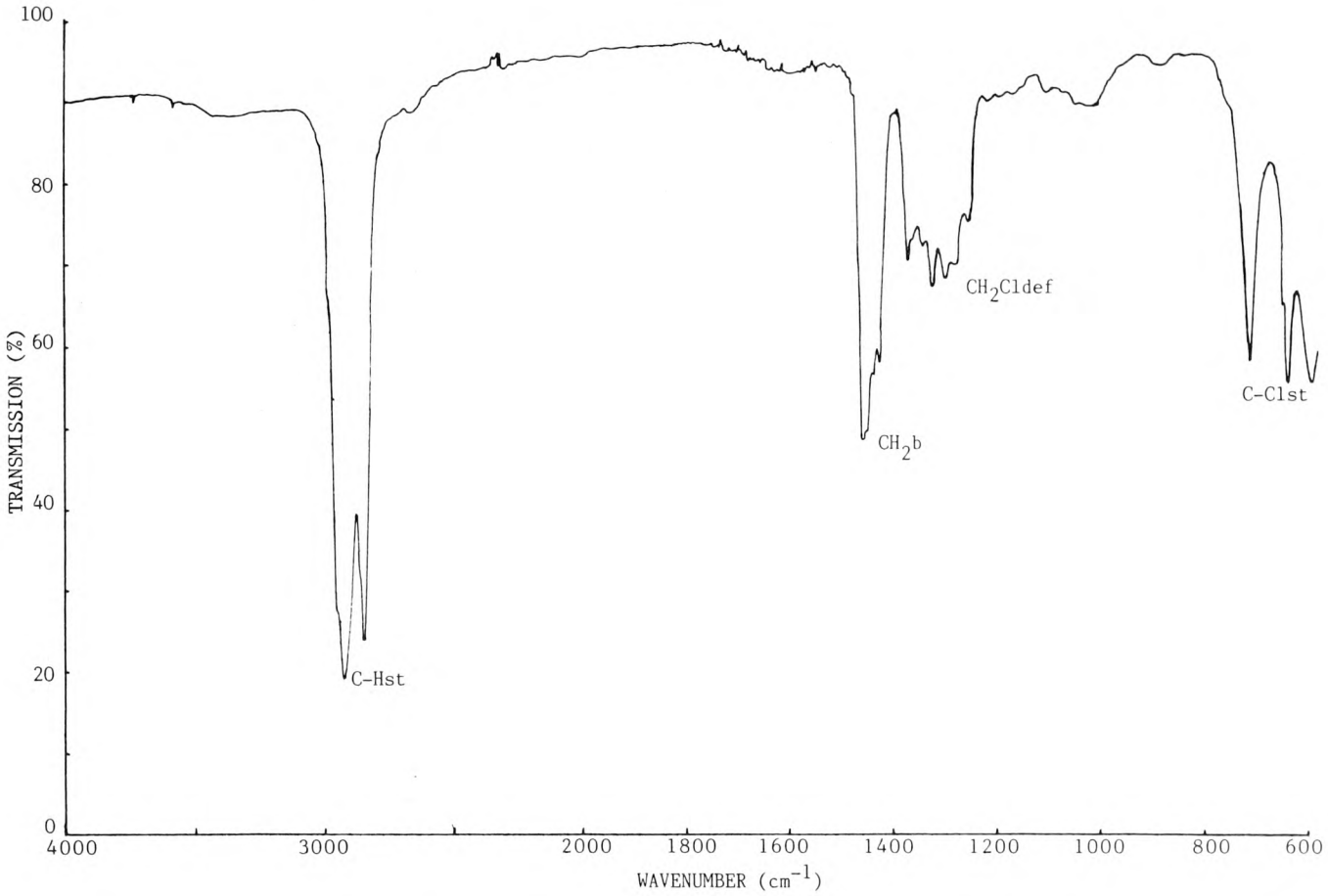
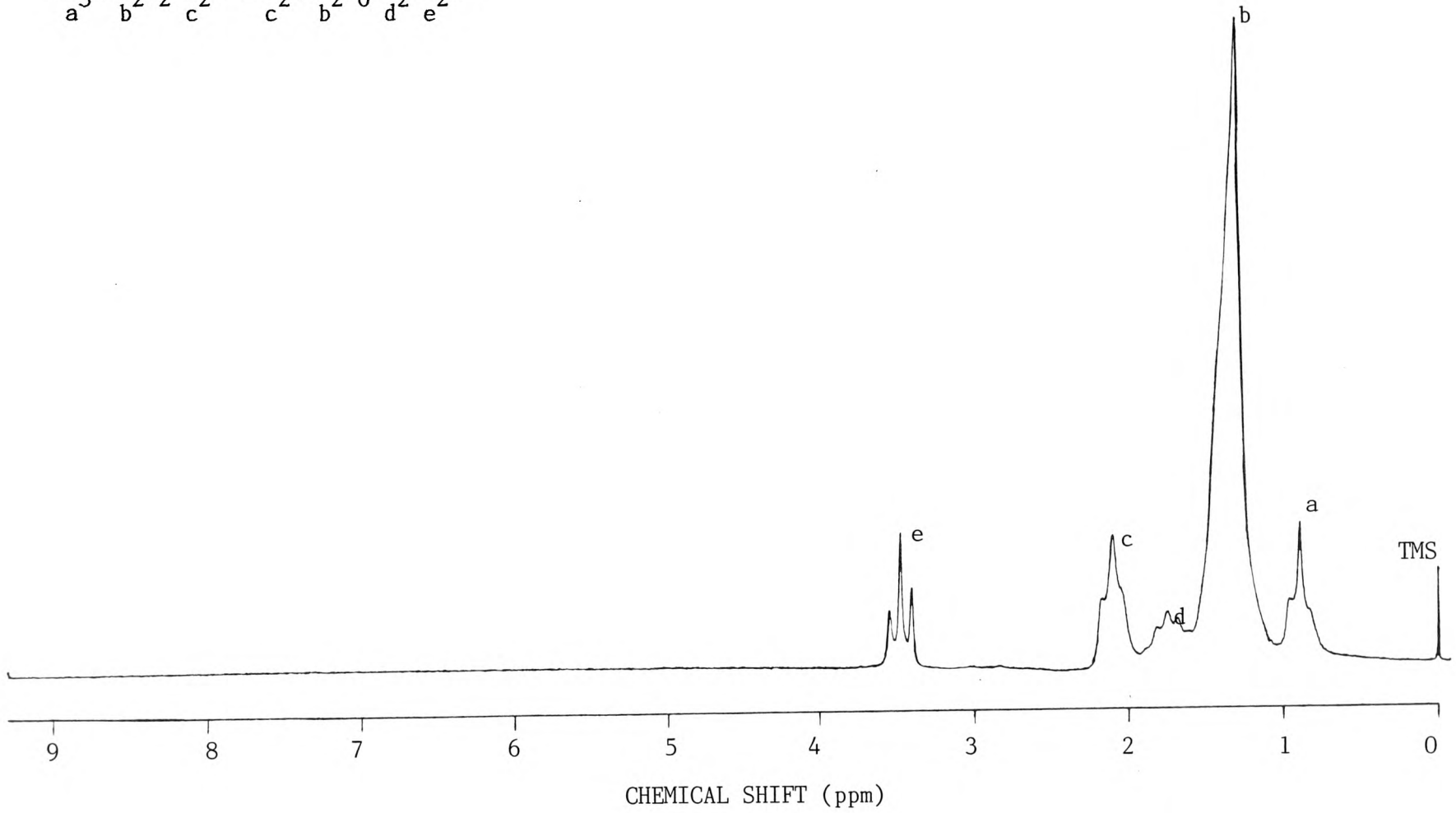


FIGURE 14 ^{1}H NMR Spectrum of 1-Chloro-10-pentadecyne

$$\underset{a}{CH_3}\underset{b}{(CH_2)_2}\underset{c}{CH_2}C{\equiv}C\underset{c}{CH_2}\underset{b}{(CH_2)_6}\underset{d}{CH_2}\underset{e}{CH_2}Cl$$



Position of Unsaturation	Chemical Shift (ppm)
(n-3)	1.11
(n-4)	0.96
(n-5)	0.92
(n-6)	0.90
(n-≧7)	0.88

Furthermore, whereas the methyl absorption in the majority of 1-chloro-alkynes is a distorted triplet, when the triple bond is (n-3) and (n-4), it is well defined.

The chemical shift of the propargylic protons, when uninfluenced by any other source tends towards 2.08-2.09 ppm. The most pronounced effect that the position of unsaturation has on this absorption is exhibited by the 4- and 5- isomers. The chemical shifts in these isomers are down-field (2.18 and 2.12 ppm respectively) of the shifts in an isolated system. This is because the propargylic protons on either side of the triple bond exhibit slightly different chemical shifts as a result of the long range deshielding of the chloride. However, the signals at 90 MHz are not sufficiently resolved that they may be regarded as separate and what is observed is an average chemical shift.

Protons on the carbon β to the chloride exhibit a chemical shift at 1.68-1.70 ppm when uninfluenced by deshielding effects of any other functional group. The absorption shifts downfield as the acetylenic bond approaches the chloride. In the 4- isomer the chemical shift is 1.82 ppm. In certain isomers however, particularly the 5- and 6- isomers, this signal is fully or partially obscured by what may be termed anomalous absorptions. These are attributable to alkyl chain protons and arise because of shifts induced by long range deshielding of the triple bond, and the chloride and methyl groups.

When uninfluenced by the deshielding effects of any functional group, the chemical shift of a methylene proton in a long alkyl chain tends towards 1.255 ppm. However, the chemical shift of a specific polymethylene proton is dependent on the deshielding it experiences as a result of functional groups. The extent of deshielding can be quite considerable in protons adjacent to a functional group, but smaller effects can extend further causing subtle changes in the basic chemical shift value of the methylene protons of the alkyl chain.

For isomeric 1-chloroalkynes, this results in a number of small absorptions between 1.25 and 1.75 ppm. As substituent effects are additive, the extent of these absorptions in some isomers, can be quite considerable, fully or partially obscuring the signal attributable to protons β to the chloride. In others (e.g. 1-chloro-10-pentadecyne, Figure 14), the anomalous shifts induced by functional groups are barely discernible as a shoulder on the main polymethylene proton signal in the expanded spectrum. In the shorter chain length compounds (C_{10}-C_{14}), several absorptions of equal intensities between 1.6 and 1.2 ppm may be apparent. Such absorptions are also exhibited in the ^1H NMR spectra of monounsaturated fatty acids.

The main difference in the ^1H spectra of the chloro- and iodo- derivatives isolated, is in the chemical shift of protons on carbons α to the halogen substituent. In both compounds, the absorption is a well defined triplet. In 1-iodoalkynes however, its chemical shift (3.16 ppm) is about 0.3 ppm upfield of the shift in the corresponding 1-chloroalkyne. ^1H NMR chemical shifts and assignments of the 1-chloroalkynes are summarised in Table 25. For convenience, the nomenclature used for the description of a fatty acid is employed. Thus, 1-chloro-7-pentadecyne

TABLE 25

^{1}H NMR Chemical Shifts and Assignments of 1-Chloroalkynes

$$\underset{a}{CH_3}\underset{\ell}{(CH_2)_{n-1}}\underset{c}{CH_2}C{\equiv}C\underset{c}{CH_2}\underset{\ell}{(CH_2)_{m-2}}\underset{d}{CH_2}\underset{e}{CH_2}Cl$$

1-Chloroalkyne[a]	Shift and Assignment (ppm)				
	a	*ℓ*[b]	*c*	*d*	*e*
10:1(7)a	1.11	1.47	2.11	1.71	3.52
11:1(4)a	0.88	1.30/1.40	2.18	1.80	3.60
(5)a	0.89	1.41	2.12	1.74[c]	3.54
(6)a	0.92[d]	1.40/1.48	2.10	1.67[c]	3.52
(7)a	0.95[d]	1.46	2.09	1.70	3.51
12:1(7)a	0.92[d]	1.41[e]/1.46	2.10	1.71	3.52
13:1(4)a	0.88	1.29/1.40[e]	2.19	1.82	3.61
(5)a	0.88	1.30/1.37	2.12	1.75[c]	3.54
(6)a	0.88	1.37	2.10	1.65[c]	3.51
(7)a	0.90	1.43	2.10	1.72	3.53
(9)a	0.96[d]	1.41	2.08	1.69	3.50
(10)a	1.10[d]	1.38	2.10	1.67	3.51
14:1(11)a	1.11[d]	1.31/1.40	2.10	1.68	3.52
15:1(4)a	0.88	1.27/1.40[e]	2.18	1.82	3.61
(5)a	0.88	1.28/1.39[e]	2.12	1.75[c]	3.55
(6)a	0.88	1.32	2.10	1.60[c]	3.51
(7)a	0.89	1.29/1.43[e]	2.09	1.70	3.52
(9)a	0.90[d]	1.40	2.08	1.67	3.50
(10)a	0.92[d]	1.37	2.10	1.68	3.52
(11)a	0.95	1.34/1.40[e]	2.09	1.67	3.51
16:1(7)a	0.88	1.28/1.44[e]	2.09	1.70	3.50
(11)a	0.93[d]	1.31[e]/1.41	2.08	1.66	3.50 3.17[f]
17:1(6)a	0.88	1.27/1.39[e]	2.11	1.62[c]	3.53
(7)a	0.88	1.28/1.41[e]	2.10	1.68	3.51
(9)a	0.89	1.38	2.09	1.67	3.50
(11)a	0.91[d]	1.30/1.40[e]	2.09	1.66	3.50
(13)a	0.96[d]	1.29/1.41[e]	2.08	1.66	3.52
18:1(7)a	0.88	1.27/1.44[e]	2.10	1.69	3.50
(11)a	0.89	1.34	2.08	1.67	3.50
(13)a	0.92[d]	1.28/1.40[e]	2.09	1.66	3.50
19:1(9)a	0.88	1.34	2.10	1.67	3.50
(10)a	0.88	1.32	2.09	1.70	3.51 3.16[f]
(11)a	0.88	1.34	2.09	1.67	3.52 3.15[f]
(13)a	0.91	1.35	2.10	1.68	3.51

FOOTNOTES

a) Nomenclature refers to Chain Length:Degree of Unsaturation (Position of Unsaturation)acetylenic Bond.
b) Denotes shift of the main polymethylene proton absorption and anomalous absorptions induced by the deshielding of functional groups.
c) Approximate shift. Absorption partially obscured by anomalous absorptions of alkyl chain methylene protons.
d) Signal is a well defined triplet.
e) Shoulderon main polymethylene absorption.
f) Resonance of α-CH_2 in the 1-iodoalkynes.

for example is designated 15:1(7)a.

^{13}C NMR chemical shifts of 1-chloroalkynes and the more salient shifts of some 1-iodoalkynes are summarised in Table 26. The spectrum of 1-chloro-5-tridecyne is illustrated in Figure 15. The shifts are based on information discussed in previous sections for α,ω-dichloroalkanes, α-chloro-ω-iodoalkanes and 1-alkynes, previously reported shifts for dialkylacetylenes,[143,144,145] and on the assumption that the influence of a functional group on the chemical shift of neighbouring carbon atoms is consistent through a range of structures. In some of the longer chain length compounds, as some of the absorptions apply to more than one carbon atom (principally chemical shifts of the alkyl chain carbons in the 29-30 ppm region), their assignment may be somewhat ambiguous.

In most 1-chloroalkynes, the two acetylenic carbon atoms show different chemical shifts around 80 ppm. In 1-chloroalkynes of general formula $CH_3(CH_2)_nC_b{\equiv}C_a(CH_2)_mCl$, the closer the triple bond to the chloride, the greater the shift between C_a and C_b. As distance between the triple bond and chloride increases, the nonequivalence decreases and eventually, in the longer chain length compounds, the signals merge. This non-equivalence may be demonstrated by the 4- to 9- 1-chloropentadecynes as summarised below.

1-Chloropentadecyne	Nomenclature	Chemical Shift (ppm)		
		C_b	C_a	C_b-C_a
4-pentadecyne	15:1(4)a	81.49	77.71	3.78
5-pentadecyne	15:1(5)a	81.00	79.18	1.82
6-pentadecyne	15:1(6)a	80.67	79.57	1.10
7-pentadecyne	15:1(7)a	80.45	79.82	0.63
8-pentadecyne	15:1(8)a	-	-	-
9-pentadecyne	15:1(9)a	80.26	80.02	0.24

This nonequivalence may be explained in terms of electric field effects

TABLE 26

^{13}C NMR Chemical Shifts and Assignments of 1-Chloroalkynes

1-Chloroalkyne[a]	Shift and Assignment (ppm)																		
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}
10:1(7)a	44.94	32.64	26.44	28.28	28.79	18.74	79.13	81.93	12.48	14.39	–	–	–	–	–	–	–	–	–
11:1(4)a	43.09	32.10	16.19	77.80	81.48	18.75	29.12	28.58	31.47	22.62	14.09	–	–	–	–	–	–	–	–
(5)a	44.57	31.74	26.36	18.18	79.18	81.09	18.74	28.90	31.12	22.15	14.00	–	–	–	–	–	–	–	–
(6)a	44.83	32.37	26.23	28.52	18.72	79.55	80.53	18.52	31.38	22.03	13.66	–	–	–	–	–	–	–	–
(7)a	44.95	32.64	26.39	28.12	28.74	18.72	79.93	80.27	20.80	22.60	13.47	–	–	–	–	–	–	–	–
12:1(7)a	44.90	32.69	26.55	28.14	29.04	18.74	79.77	80.31	18.52	31.41	22.03	13.66	–	–	–	–	–	–	–
13:1(4)a	43.00	32.11	16.16	77.78	81.46	18.74	29.14	28.89	29.33	29.33	31.94	22.72	14.09	–	–	–	–	–	–
(5)a	44.56	31.70	26.35	18.13	79.16	81.01	18.74	29.21	28.87	28.87	31.87	22.69	14.10	–	–	–	–	–	–
(6)a	44.82	32.34	26.22	28.50	18.64	79.56	80.66	18.74	29.14	28.57	31.39	22.52	14.09	–	–	–	–	–	–
(7)a	44.95	32.65	26.53	28.06	28.89	18.69	79.82	80.43	18.74	28.89	31.06	22.25	14.00	–	–	–	–	–	–
(9)a	44.07	32.65	26.82	28.76	28.89	28.76	29.16	18.76	80.23	80.09	20.81	22.62	13.47	–	–	–	–	–	–
(10)a	44.98	32.64	26.85	28.13	29.20	29.13	28.87	29.13	18.77	79.50	81.65	12.30	14.43	–	–	–	–	–	–
14:1(11)a	44.87	32.64	26.94	28.84	29.13	29.43	29.43	28.95	29.13	18.72	79.40	81.81	12.45	14.40	–	–	–	–	–
15:1(4)a	43.08	32.08	16.20	77.81	81.49	18.74	29.18	28.84	29.18	29.35	29.35	29.40	31.94	22.69	14.10	–	–	–	–
(5)a	44.56	32.74	26.38	18.19	79.18	81.00	18.79	29.21	28.94	29.21	29.35	29.35	31.94	22.72	14.09	–	–	–	–
(6)a	44.83	32.28	26.21	28.50	18.64	79.57	80.67	18.74	29.21	28.89	29.21	29.33	31.92	22.72	14.10	–	–	–	–
(7)a	44.88	32.14	26.28	28.15	28.65	18.64	79.79	80.40	18.70	29.18	28.84	29.04	31.84	22.69	14.10	–	–	–	–
(9)a	44.95	32.72	26.89	28.94	29.04	28.75	29.16	18.76	79.97	80.18	18.76	28.86	30.43	22.30	14.01	–	–	–	–
(10)a	45.02	32.60	26.82	28.72	29.23	29.13	28.77	29.13	18.69	80.03	80.16	18.37	31.23	22.17	13.64	–	–	–	–
(11)a	45.05	32.69	26.94	28.91	29.45	29.21	29.21	28.91	29.14	18.78	80.28	79.99	20.81	22.62	13.47	–	–	–	–
16:1(7)a	44.90	32.56	26.50	28.04	28.69	18.66	79.83	80.41	18.72	29.21	28.89	29.21	29.21	31.94	22.72	14.09	–	–	–
(11)a	44.98	32.63	36.82	28.79	29.31	29.31	29.26	28.79	29.14	18.74	79.92	79.92	18.42	31.26	21.86	13.64	–	–	–
b	7.09	33.47	30.38	29.04															
17:1(6)a	44.84	32.33	26.21	28.51	18.68	79.57	80.65	18.78	29.18	28.89	29.18	29.64	29.64	29.40	31.94	22.69	14.10	–	–
(7)a	45.05	32.49	26.35	27.96	28.77	18.62	79.75	80.38	18.69	29.09	28.77	29.09	29.09	29.40	31.82	22.60	14.08	–	–
(9)a	45.05	32.69	26.89	28.77	28.89	28.77	29.16	18.79	80.09	80.31	18.79	29.16	28.89	29.04	31.84	22.67	14.11	–	–
(11)a	45.02	32.67	26.86	28.89	29.36	29.36	29.18	28.54	29.18	18.74	80.15	80.16	18.74	28.89	30.53	22.59	14.00	–	–
(13)a	44.90	32.72	26.91	28.90	29.13	29.44	29.44	29.44	29.13	28.84	29.13	18.77	80.15	79.84	20.81	22.62	13.46	–	–
18:1(7)a	44.85	32.56	28.38	28.01	28.92	18.66	79.79	80.41	18.74	29.26	28.92	29.26	29.54	29.54	29.39	31.94	22.67	14.10	–
(11)a	45.02	32.67	26.87	28.84	29.36	29.36	29.18	28.84	29.18	18.77	80.15	80.15	18.77	29.18	28.50	30.41	22.60	14.09	–
(13)a	44.90	32.65	26.94	28.29	29.13	29.44	29.44	29.44	29.13	28.84	29.13	18.77	80.18	80.18	18.41	31.28	22.11	13.63	–
19:1(9)a	44.98	32.65	26.82	28.77	29.26	28.89	29.16	18.76	80.00	80.21	18.76	29.16	28.89	29.26	29.26	29.39	31.94	22.67	14.09
(10)a	44.97	32.70	26.92	28.84	29.33	29.23	28.89	29.23	18.76	80.06	80.18	18.76	29.23	28.78	29.33	29.40	31.94	22.69	14.10
b	6.98	33.59	30.51	29.18															
(11)a	45.00	32.67	26.89	28.84	29.42	29.28	29.11	28.84	29.18	18.77	80.11	80.21	18.77	29.28	28.84	29.11	31.77	22.62	14.10
b	6.98	33.55	30.48	29.11															
(13)a	45.05	32.66	26.90	28.74	29.53	29.53	29.45	29.45	29.19	28.89	29.19	18.76	80.12	80.12	18.76	28.74	30.98	22.35	14.00

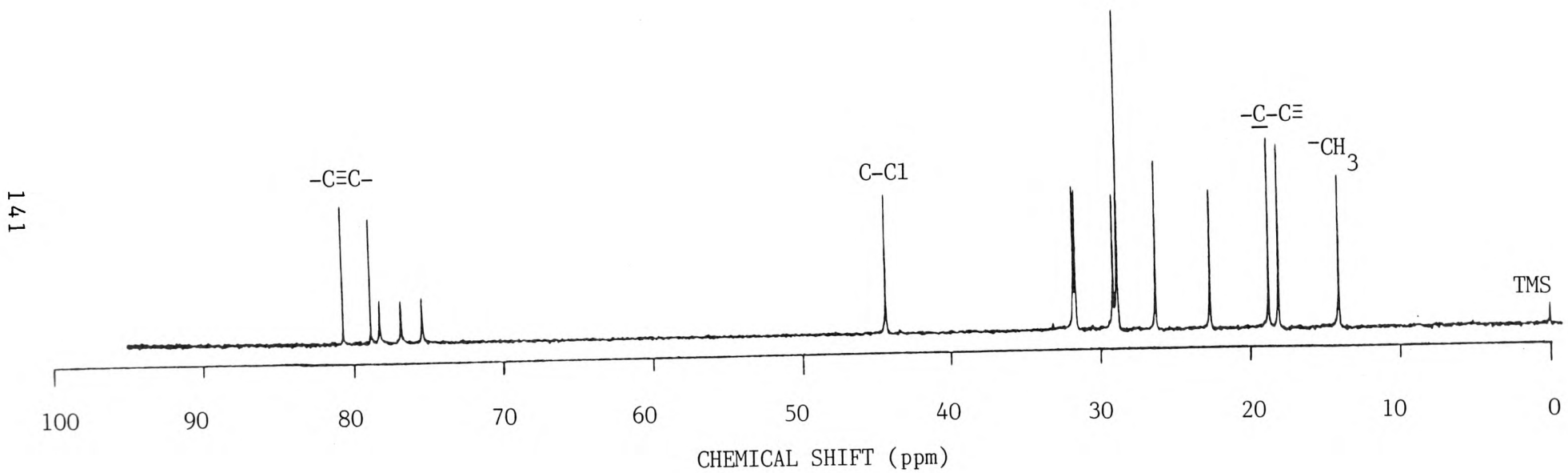
FOOTNOTES

a) Nomenclature refers to Chain length:Degree of unsaturation (Position of Unsaturation) acetylenic bond

b) Salient shifts of some 1-iodoalkynes

FIGURE 15 ^{13}C NMR Spectrum of 1-Chloro-5-tridecyne

$CH_3(CH_2)_6C{\equiv}C(CH_2)_4Cl$



and is discussed at length for the ^{13}C spectra of fatty acids. As the triple bond approaches the methyl group the acetylenic carbon shifts once again split. Two chemical shifts were observed in all but five of the longer chain length 1-chloroalkynes. It follows from the chemical shifts featured in Table 26 that the chloride group exerts a differential effect on a pair of acetylenic carbon atoms up to the 10/11- isomers whilst the methyl group exerts a differential effect when the triple bond is at (n-3), (n-4) and (n-5).

The propargylic carbon atoms are strongly influenced by the adjacent acetylenic system. In the absence of any influence from the primary chloride or terminal methyl groups, the chemical shift of a propargylic carbon in 1-chloroalkynes tends towards 18.74 ppm. However, this shift is modified by the chloride and methyl groups so there are occasionally two signals. This nonequivalence (which may again be explained in terms of electric field effects), is not generally as pronounced as that exhibited between the acetylenic carbons with the following exceptions. Propargylic carbons shift upfield by about 2 ppm when β to the chloride e.g. 1-chloro-5-pentadecyne (16.20 ppm) and downfield by about 2 ppm when β to the methyl group e.g. 1-chloro-9-tridecyne (20.81 ppm). When the propargylic carbon is α to the methyl group, there is an upfield shift of around 6 ppm e.g. 1-chloro-11-tetradecyne (12.45 ppm). It is possible from these easily assigned shifts to identify 1-chloroalkynes when unsaturation is in the 4- to 7- and (n-3) to (n-5) position.

The chemical shift for the terminal methyl carbon when isolated is 14.09-14.10 ppm. As the triple bond approaches the methyl group, this absorption shifts upfield. The chemical shift in (n-6), (n-5) and (n-4) isomers is 14.00, 13.65 and 13.47 ppm respectively. In contrast however,

when unsaturation is (n-3), a downfield shift of about 0.3 ppm is observed (14.40 ppm).

For most 1-chloroalkynes, the chemical shift of the carbon α to the chloride lies between 44.56 and 45.19 ppm. In the 4- isomers however, an upfield shift of 1.5-2.0 ppm is observed and the signal absorbs at about 43.00 ppm. Substituent effects on the carbons of the polymethylene chains are normally in the region of 1 ppm and these signals generally absorb between 28.64 and 29.74 ppm.

SECTION TWO

THE SYNTHESIS OF LONG CHAIN MONOUNSATURATED FATTY ACIDS

1-Chloroalkynes were converted to the *cis* and *trans* alkenoic acids via the aetylenic acids and partial reduction of the acetylenic bond. As some acetylenic acids are known to occur in nature,[13] the discussion of their synthesis in this Section is appropriate.

Before discussing the synthesis of fatty acids from the intermediate 1-chloroalkynes however, it would be appropriate at this stage to briefly review some of the problems and practical considerations that have to be borne in mind when handling fatty acids and lipid samples in general.

5 Some Practical Considerations in the Handling of Lipid Samples

5.1 The Problem of Oxidation

It is well known that the oxidation of olefinic compounds by atmospheric oxygen readily occurs unless adequate precautions are taken to prevent such occurrence. In edible fats and oils, such processes are important in the development of rancidity and off-flavours and is sometimes significant in the polymerisation of highly unsaturated oils.

The oxidation of unsaturated fatty acids may be broadly categorised as enzymic and non-enzymic. Non-enzymic oxidation may be further subdivided into autoxidation and photo-oxidation. In the context of lipids such as dietary lipids and adipose tissue, all three methods of oxidation may be influential. In the context of the synthesised acids however, only autoxidation is an important factor, as as enzymes and photosensitisers, which are responsible for inducing enzymic and photo-oxidation in natural lipids, are absent. All three forms of oxidation are now briefly summarised.

In all three cases, the first isolatable oxidation products are unsaturated hydroperoxides although the actual structures depend on the mode of oxidation. These hydroperoxides then undergo further reaction to furnish more extensively oxidised derivatives of the original alkene, compounds of lower molecular weight following fission of the carbon chain and, compounds of higher molecular weight (dimers and polymers).

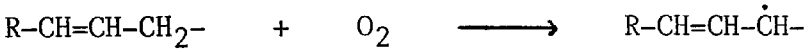
a) Enzymic Oxidation

The enzymic formation of hydroperoxides is brought about by enzymes such as lipoxygenase which catalyses the interaction between oxygen and linoleic acid (or certain other polyunsaturated acids). Such enzymes are widely distributed in the plant kingdom and also exist in animals. An enzyme preparation from soybean (lipoxygenase I) has been extensively studied.[164]

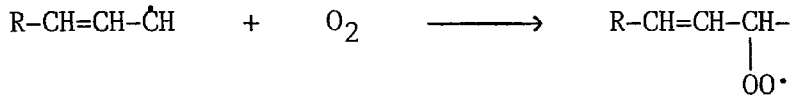
b) Autoxidation

The major non-enzymic oxidation process is known as autoxidation. When unsaturated lipids are exposed to atmospheric oxygen, there is initially an induction period during which any antioxidants present are consumed and then free radicals begin to accumulate. This period is then followed by rapid oxygen absorption and an autocatalytic radical reaction (autoxidation) sets in.[165]

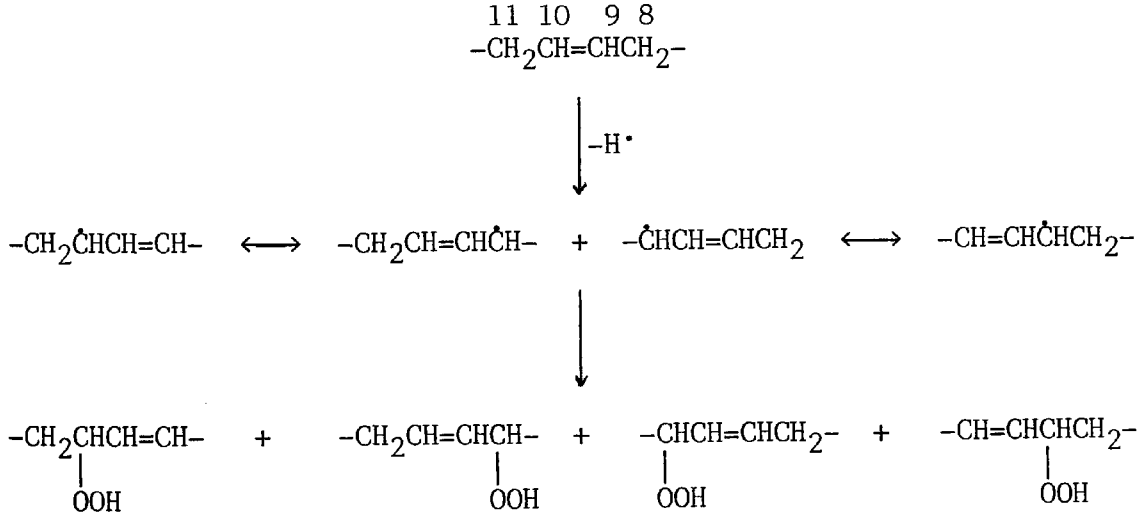
The mechanism and pathways of autoxidation are complex but are known to involve initiation, propagation and termination steps. The nature of the initiation reaction is still uncertain although it is widely accepted that it involves the formation of radicals by abstraction of a hydrogen atom at an allylic carbon. In addition, hydroperoxides once formed, furnish additional initiating radicals.



The propagation sequence involves the production of a radical from the alkene, and its subsequent reaction with oxygen to form a hydroperoxide.

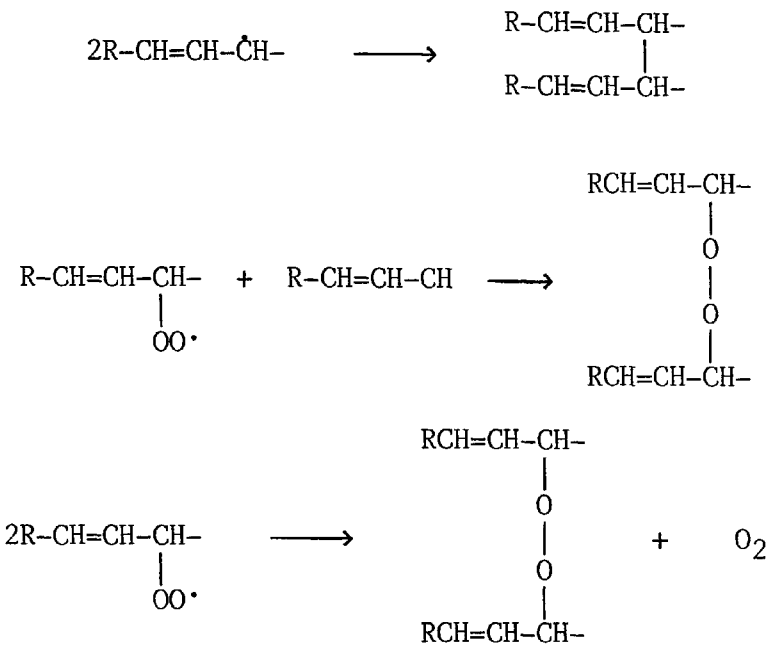


The radical, produced by reaction at the allylic position is resonance stabilised and this effects the structure of the reaction products. The hydroperoxides produced from the autoxidation of methyl oleate for example, are a mixture of the *cis* and *trans* isomers of 8-hydroperoxy-9-, 9-hydroperoxy-10-, 10-hydroperoxy-8- and 11-hydroperoxy-9-octadecenoates. The formation of these compounds is explained in terms of the propagation sequence which occurs via two resonance stabilised-allyl radicals.



Termination reactions involve the interaction of radicals to produce non-initiating and non-propagating products. Such reactions have not

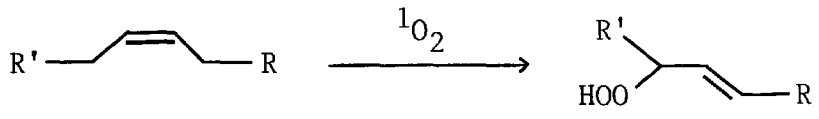
been extensively studied.



Susceptibility to autoxidation increases with increasing unsaturation. Methyl linoleate reacts 10-40 times faster than oleate because of the enhanced reactivity of the C-11 methylene group lying between two double bonds.[166]

c)Photo-oxygenation

In the process of photo-oxygenation, oxygen is converted to its more reactive state, usually in the presence of a suitable sensitiser such as chlorophyll or erythrosine. This reacts with alkenes by a concerted mechanism not involving a radical and is accompanied by double bond migration. Furthermore, it has no induction period, is unaffected by antioxidants but is inhibited by singlet oxygen quenchers such as carotene.[167]



The hydroperoxides produced differ from those obtained in autoxidation. Methyl oleate for example, yields 9-hydroperoxy-10- and 10-hydroperoxy-8-octadecenoate only. Other sensitised photolytic reactions, for example with riboflavin produce alkene radicals which give the same products as direct autoxidation.

The relative rates of reaction of monoenes, dienes and trienes with this more reactive oxygen species (1:1.3:2.3 at 37°C) are very different from the relative rates of autoxidation (1:27:77 at 37°C).

5.2 General Practical Precautions Against Autoxidation

Usually, autoxidation of pure monoenoic acids (such as those synthesised here) at room temperature is a slow reaction occurring only after a long induction period. The induction period is shortened and the subsequent rate of autoxidation increased by any one of several factors including increase in temperature, irradiation, use of non-polar solvents, increasing surface:volume ratio and catalysts such as Cu, Fe, Mn and other transition metals.[168] Furthermore, monoenoic acids in mixtures containing PUFA e.g. dietary lipids also have shorter induction periods because the more readily formed products of polyunsaturated oxidation can initiate monoenoic oxidation which is invariably accompanied by isomerisation into *trans* components.

As a result of the particular nature of previous[55,57,58,60] and future investigations in which *trans* acids are used as one of the differentiating parameters, it is necessary that all possible precautions against oxidation (and accompanying isomerism) be taken.

Necessarily therefore, lipid samples (particularly those with a high degree of unsaturation) whenever possible should be handled in an

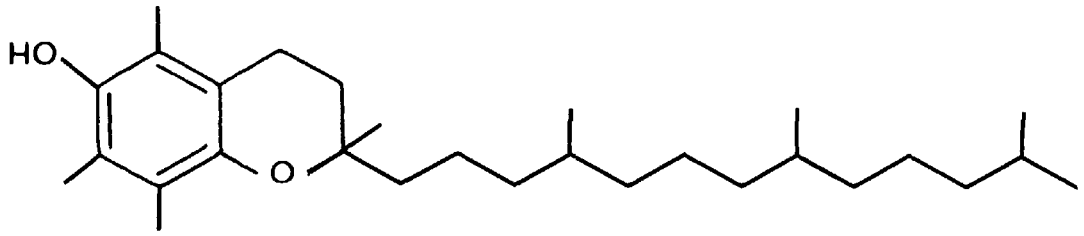
atmosphere of nitrogen. Furthermore, all solvents used in the preparation and handling of lipid samples should be flushed with nitrogen to displace any dissolved oxygen. Autoxidation may be further retarded by the addition of antioxidants (Figure16). Antioxidant properties are exhibited by a variety of chemical structures which include natural e.g. tocopherols, and synthetic e.g. BHT, compounds. The mode of action may involve either reaction of the oxidising agent with the antioxidant in lieu of the lipid, or a chain reaction termination in which radical intermediates, required for the propagation of autoxidation react with the antioxidant thereby interrupting propagation. Antioxidants therefore possess the capacity to donate hydrogen atoms readily or the capacity to react directly with the reactive radical to produce an unreactive adduct.

Although in lipid samples from natural sources, natural tissue antioxidants such as tocopherols may afford some protection, the addition of an additional synthetic antioxidant is recommended. Suitable compounds are phenolic compounds such as 2,6-di-*tert* butyl p-cresol (BHT) or *tert*-butylhydroquinone (BHQ) which should be added at a level of 0.05%.[169] Previous studies have indicated that the use of BHT results in spurious GLC peaks that could be taken as 16:1 or 14:0 according to the stationary phase used.[170] BHQ however has been shown to give no artefacts and no perceptible elevation of baseline in GLC under the employed conditions. Both BHT and BHQ may be removed if required by volatisation in a stream of nitrogen during solvent evaporation.

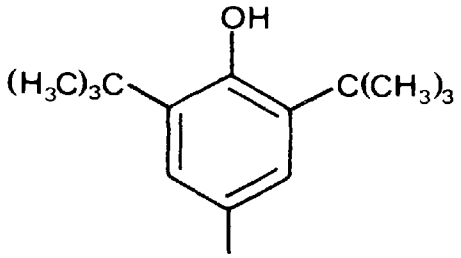
Large volumes of solvents were removed from lipid samples in a rotary film evaporator at 30°C. Evaporation of small volumes of solvents was effected via a stream of nitrogen gas directed over the surface of the

FIGURE 16 Chemical Structures of Some Naturally Occuring and Synthetic Antioxidants

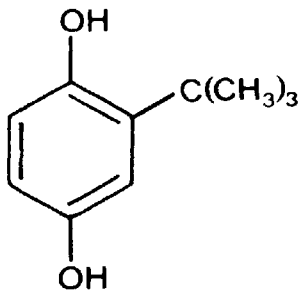
a) α-Tocopherol



b) 2,6-Di-*tert*-butyl-p-cresol (BHT, Butylated hydroxytoluene)



c) 1,4-Dihydroxy-2-*tert*-butyl Benzene (BHQ, Butylhydroquinone)



solution kept in a warm water bath (30°C). It should be emphasised however that over-vigorous application of such evaporation techniques can cause some loss of material which may normally be considered as involatile, in particular, methyl esters of medium chain fatty acids up to and including C_{14}. Accordingly, appropriate care should be exercised when preparing samples containing such acids on a quantitative basis.

As an added precaution, it became standard practice never to leave any lipid extract or derivative in a dry state, and they were immediately taken up and stored in inert and purified polar solvents (e.g. chloroform or carbon disulphide). For long term storage, they were kept at -20°C, under nitrogen, in sealed glass screw capped bottles. No deterioration was apparent in the synthesised acids when stored in this manner.

5.3 General Practical Precautions Against Contamination

a) Solvents

All solvents, including high purity analytical grades, contain traces of impurities some of which have been deliberately added by the manufacturers. As large volumes of solvents are often required for the isolation of very small amounts of lipids, serious contamination can occur. Accordingly therefore, all solvents used in the preparation and handling of acids and acid derivatives were prepared by appropriate methods to a high degree of purity. Such procedures invariably involved at least distillation and as such, the use of an efficient fractionating column was essential. This consisted of a glass column (50x2.5cm) packed with stainless steel gauze around which was a heat insulating vacuum sealed jacket. All openings to the atmosphere in the solvent still were protected by guard tubes containing silica gel.

b) Other Contaminants

Apart from the potential contaminants in solvents, extraneous lipid-like materials may arise from a variety of sources. Accordingly therefore, all contact with extraneous oils, greases, plastics (other than that made of Teflon) and plasticisers were avoided. In particular, the use of rubber and plastisicised tubing during solvent evaporation in a stream of nitrogen was avoided and short lengths of neoprene tubing was found to be satisfactory. The potential sources of contamination have been reviewed.[171]

c) Glassware

Glassware was cleaned overnight by soaking in a 2% w/v solution of Quadralene detergent (Fisons Scientific Apparatus Ltd) followed by thorough rinsing with distilled and deionised water. This was then dried in a clean, specially reserved oven at ca. 80°C and stored in either desiccators or otherwise suitably prepared contamination free cabinets. Conventional glassware with grease-free joints and groundglass stoppers was used for some chemical manipulations including simple solvent distillations. Extensive use was also made of a glassware system marketed under the trademark SVL (made by Sovirel, France, supplied by V A Howe and Co Ltd, London). It utilises a unique grease-free connecting system of butt joints held together by screw threaded thermally resistant plastic flanges, protected by PTFE coated "O" rings. In particular, the use of SVL type test tubes of 10 cm^3 and 30 cm^3 capacity with PTFE protected screw caps providing air tight seals, were found beneficial as separatory (organic and aqueous layers may be efficiently separated with the use of disposable Pasteur pippettes), reaction (hydrolysis, esterification etc) and storage vessels.

6 The Synthesis of Acetylenic Acids

1-Chloroalkynes were converted to acetylenic acids by one of two methods depending on whether chain extension by one or two carbon atoms was required.

6.1 Conversion of 1-Chloroalkynes to Acetylenic Acids via the Addition of Two Carbon Atoms

1-Chloroalkynes of chain lengths 10, 12, 14, 16 and 18 were converted into acetylenic acids via reaction with diethyl malonate.

$$CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl \xrightarrow[(2)\ -CO_2]{(1)CH_2(CO_2Et)_2} CH_3(CH_2)_nC{\equiv}C(CH_2)_mC(=O)OEt$$

Sodium ethoxide is first formed from the reaction of super dry ethanol[172] with sodium metal.

$$2C_2H_5OH + 2Na \longrightarrow 2C_2H_5O^-Na^+$$

Addition of freshly distilled diethyl malonate to this with stirring results in the formation of the enolate anion (sodio malonic ester).

$$CH_2(CO_2Et)_2 \xrightarrow{NaOEt} {}^-CH(CO_2Et)_2$$

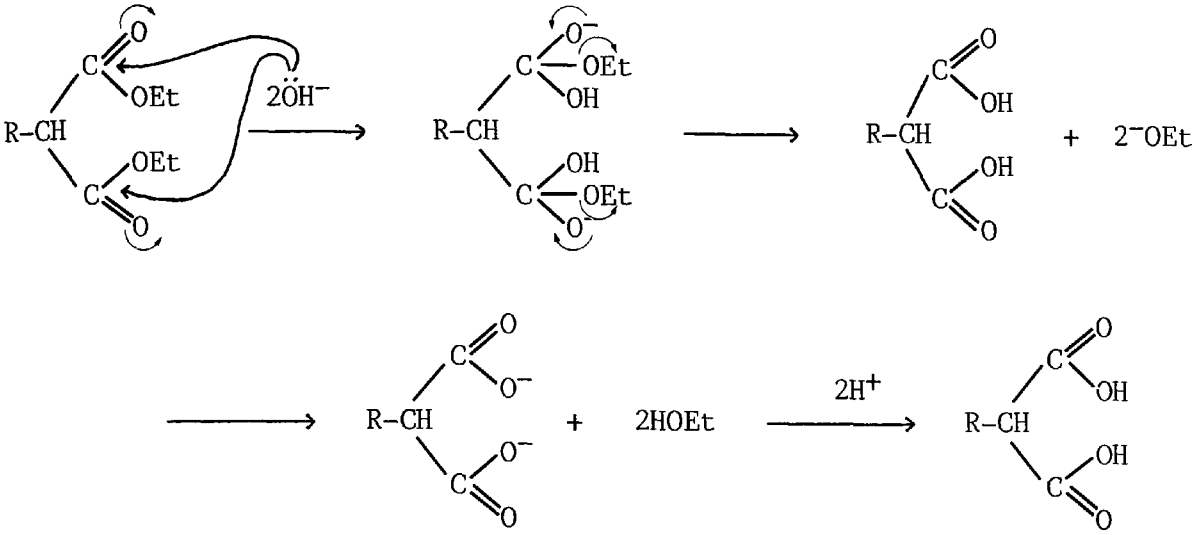
This nucleophilic anion undergoes an S_N2 reaction with a 1-chloroalkyne resulting in the formation of a *gem*-diethyl ester of a monoalkyne.

$$R\text{-}Cl + {}^-CH(CO_2Et)_2 \longrightarrow R\text{-}CH(CO_2Et)_2$$

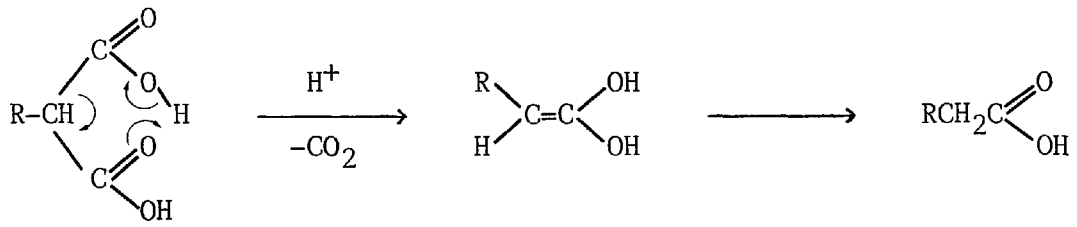
$R=CH_3(CH_2)_nC{\equiv}C(CH_2)_m$

Alkaline hydrolysis of this *gem*-diester, followed by acidification results in the formation of the *gem*-diacid derivative via essentially a nucleophilic addition/elimination reaction (also known as nucleophilic

acyl substitution).



The leaving ethoxide removes protons to form the stabler *gem* dicarboxylate anion which upon acidification yields the *gem*-diacid. This then readily undergoes decarboxylation upon heating under acidic conditions via a cyclic mechanism. The initial product is an acid enol which rapidly converts to the corresponding acetylenic acid.



The reaction is well known and has been widely manipulated for the synthesis of carboxylic acids. The reaction, when involving the use of short chain alkyl halides (C_2-C_6), occurs rapidly and vigorously and invariably requires controlling. With longer chain alkyl halides such as the 1-chloroalkynes used here, the reaction is not as spontaneous and harsher reaction conditions are necessary. This results from the relatively lower reactivity of long chain 1-chloroalkynes, arising from

the steric constraints of S_N2 reactions.

The *gem*-diesters were isolated and partially purified before their subsequent hydrolysis and decarboxylation to the acetylenic acid. For the most part, this involved the distillation of the lower boiling point contaminants (mainly starting materials) under reduced pressure to leave the desired product as residue. Yields and boiling points where recorded are summarised in Table 27. Generally, the reaction proceeded well in the case of the shorter chain length compounds but rather poorly for the longer chain length compounds.

Dominant features of the IR spectra of these compounds, in addition to ubiquitous C-H aliphatic stretching and bending, result from absorptions attributable to the ester functional groups. There is a very strong, broad absorption because of C-O-C asymmetric stretching between 1328 and 1185 cm^{-1}. This is accompanied by strong, sharp symmetric C-O-C stretching at 1036 cm^{-1} and carbonyl stretching at 1740 cm^{-1}. In addition, there is a weak but sharp absorption at 857 cm^{-1} which is characteristic of ethyl esters. Again -C≡C- stretching is effectively IR inactive. The IR spectrum of ethyl (2-carboxyethyl)-9-dodecynoate is illustrated in Figure 17.

The ^1H NMR spectra exhibit seven readily discernible absorptions, two of which may be assigned to the ester functions. These are the quartet centered on 4.16 ppm for the methylene protons of the ethoxy group and the triplet at 1.26 ppm for the methyl protons of the ethoxy group. The chemical shift of this latter signal is partially superimposed on the single absorption peak for the polymethylene protons of the hydrocarbon chain. The methine proton which neighbours the ester moieties, absorbs

TABLE 27

Yields and Boiling Points of *gem*-Diethyl Esters of Long Chain Monoalkynes of General Formula $CH_3(CH_2)_nC{\equiv}C(CH_2)_mCH(CO_2C_2H_5)_2$

Ethyl (2-carboxyethyl)-alkynoate	n	m	b.p.(°C)	mm	Yield(g)	Yield(%)[a]
-9-Dodecynoate	1	6	110-114	0.5	21.9	66.0
-9-Tetradecynoate	3	6	118-120	0.2	16.2	55.7
-13-Hexadecynoate	1	10	139-140	0.4	14.3	50.8
-9-Octadecynoate	7	6	b	-	11.9	40.2
-13-Octadecynoate	4	10	b	-	8.3	35.0
-9-Eicosynoate	9	6	b	-	3.8	29.2
-13-Eicosynoate	5	10	173-174	0.2	3.3	22.9
-15-Eicosynoate	3	12	b	-	3.5	27.0

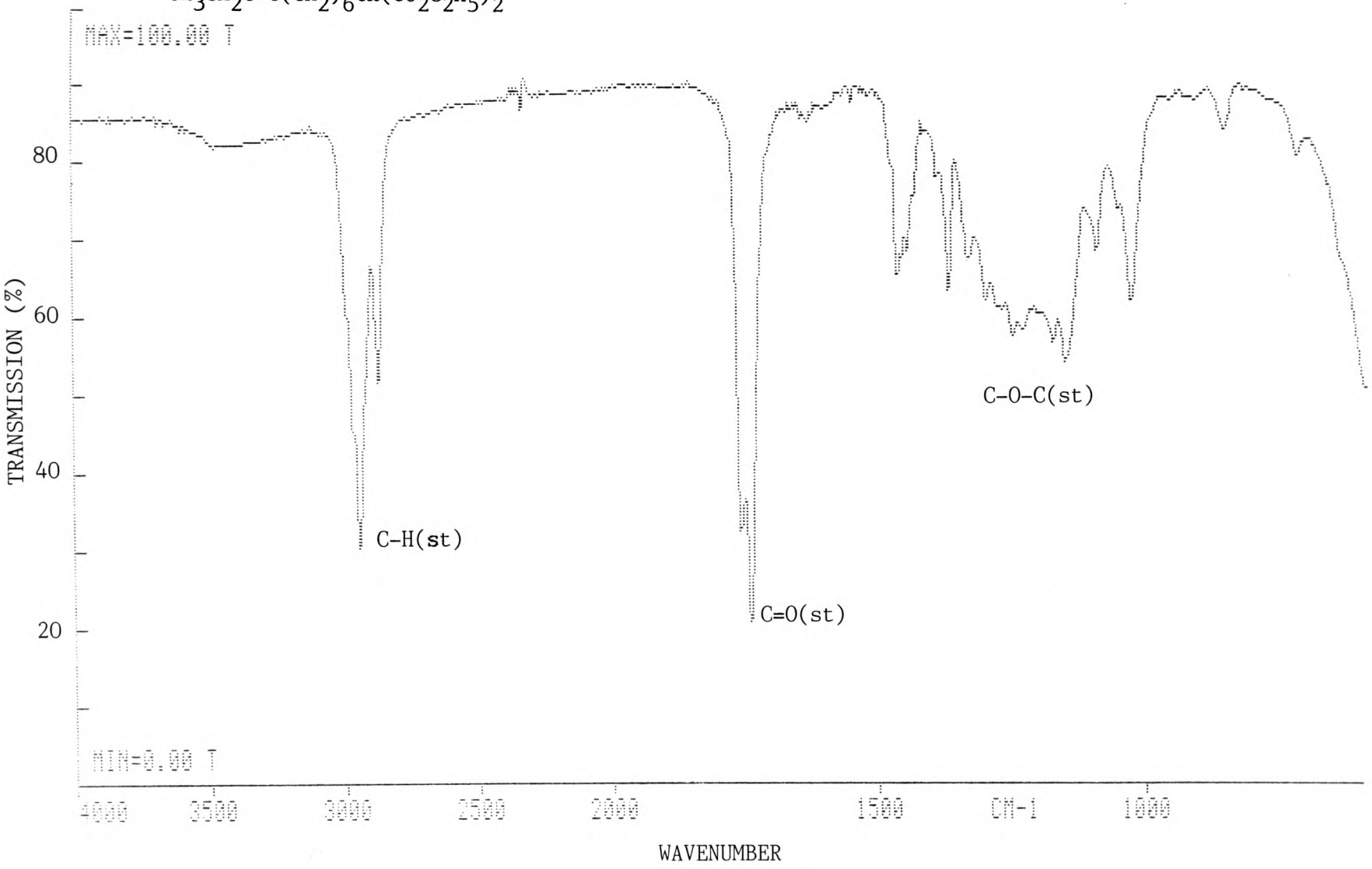
FOOTNOTES

a) Based on 1-Chloroalkyne

b) Recovered as residue from distillate.

FIGURE 17 IR Spectrum of Ethyl (2-carboxyethyl)-9-dodecynoate

$CH_3CH_2C{\equiv}C(CH_2)_6CH(CO_2C_2H_5)_2$



as a well defined triplet at 3.30 ppm and together, the chemical shifts of these three signals are anomalous. These anomalies occur as a result of the anisotropic field generated in this instance by the carbonyl groups.

The remaining signals exhibit similar chemical shifts and characteristics on increasing chain length and position of unsaturation as previously discussed for the 1-chloroalkynes. The ^{1}H NMR spectrum of ethyl (2-carboxyethyl)-9-tetradecynoate illustrated in Figure 18 is typical.

The main ^{13}C NMR chemical shifts of carbons of the monoalkyne chain are mostly analogous with those of 1-chloroalkynes. The acetylenic carbons absorb around 80 ppm and the propargylic carbons around 18 ppm except when the triple bond is (n-3) to the terminal methyl group when the shift is about 12 ppm. Furthermore, all subtle differences in shifts with respect to the position of the triple bond exhibited in the 1-chloroalkynes, are exhibited here.

The chemical shifts of the methyl and methylene of the ethoxy group are about 14.13 and 61.24 ppm respectively and the carbonyl carbon absorbs well downfield of all other signals at 169.4 ppm. C-2 is to a large extent deshielded as a result of the ester moieties and absorbs at around 52 ppm. The spectrum of ethyl (2-carboxyethyl)-9-tetradecynoate is illustrated in Figure 19.

Alkaline hydrolysis of the *gem*-diesters, followed by decarboxylation by refluxing in an aqueous acidic solution resulted in the formation of acetylenic acids. Analysis of the crude reaction product from initial runs indicated the presence of two main contaminants.

FIGURE 18 ^{1}H NMR Spectrum of Ethyl (2-carboxyethyl)-9-tetradecynoate

$CH_3(CH_2)_2CH_2C{\equiv}CCH_2(CH_2)_4CH_2CH(CO_2CH_2CH_3)_2$

a b c c b d e f g

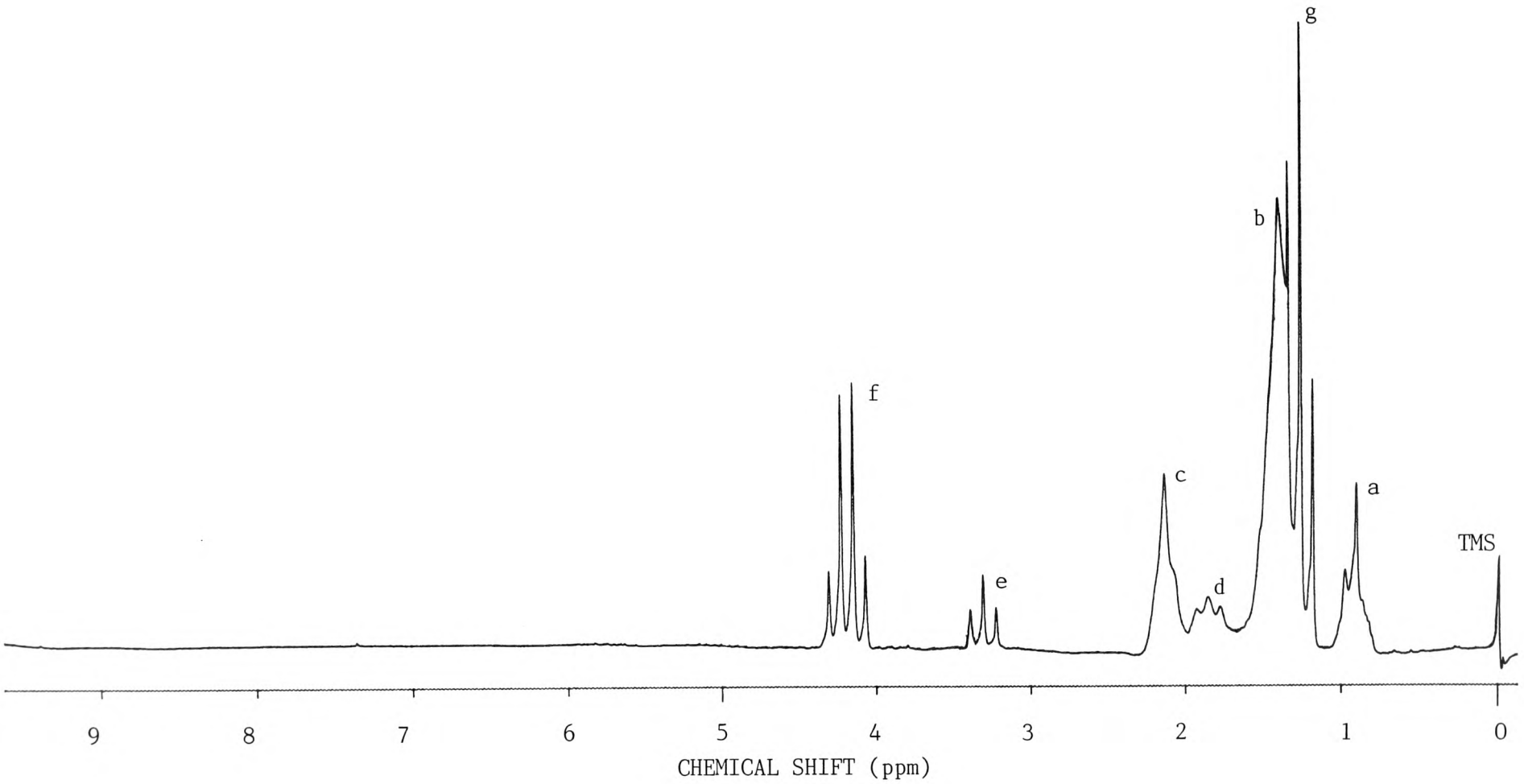
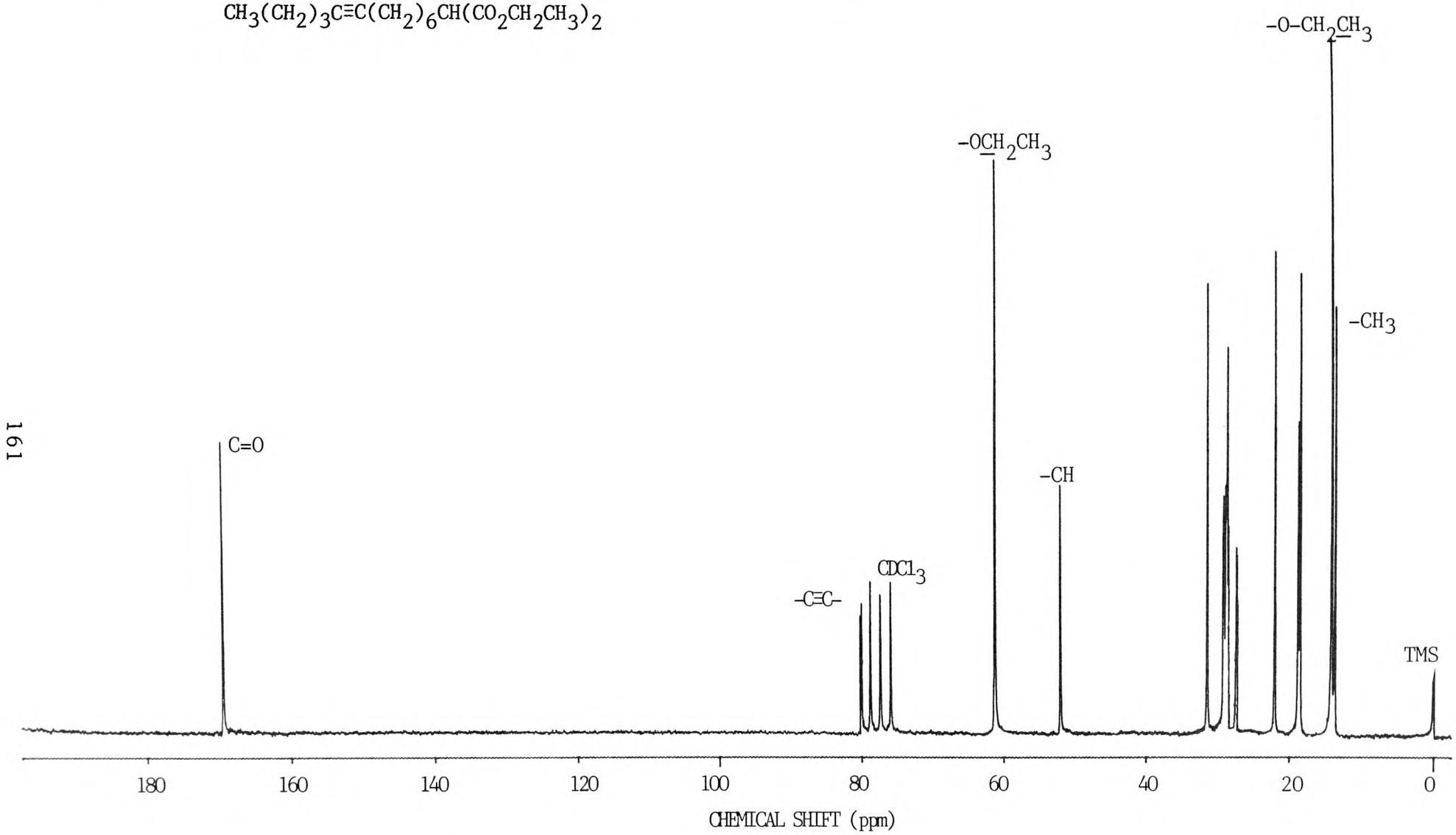
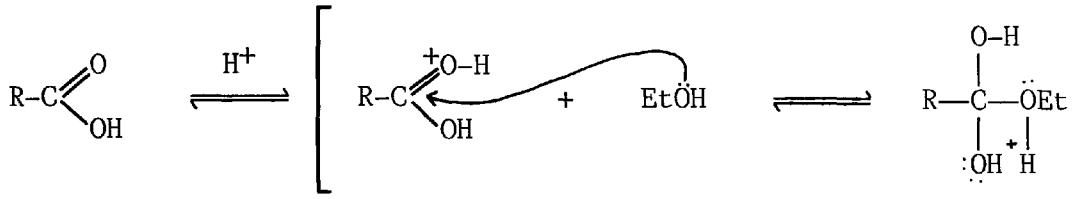


FIGURE 19 ^{13}C NMR Spectrum of Ethyl (2-carboxyethyl)-9-tetradecynoate

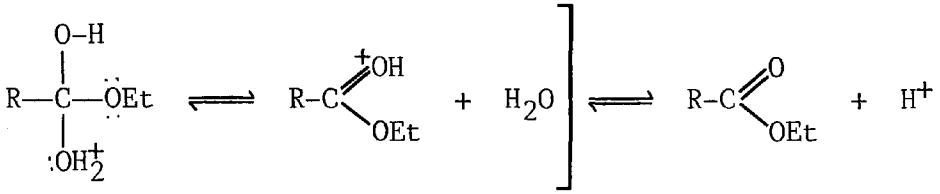
$CH_3(CH_2)_3C{\equiv}C(CH_2)_6CH(CO_2CH_2CH_3)_2$



On occasions, small amounts of ethyl alkynoates were detected. Their formation arose from the failure to remove all the ethanol (produced as a by-product during alkaline hydrolysis), from the reaction vessel prior to decarboxylation. On such occasions, the ester resulted from Fischer esterification between ethanol, and the acetylenic acid initially formed.



$R=CH_3(CH_2)_nC\equiv C(CH_2)_m$



Provided that all ethanol was removed prior to acidification, formation of the ethyl ester was eliminated. Furthermore, because of the violent and exothermic nature of the acidification procedure, it was desirable to perform this stage carefully at 0^oC to prevent possible rearrangement of the product.

The other major contaminant recovered from the initial runs was the *gem* diacid derivative which resulted from incomplete decarboxylation. These were eliminated by increasing the reaction time in subsequent runs. Those cases in which the diacid was recovered were simply resubmitted to further decarboxylation. The presence of such materials were indicated by characteristic ^{13}C NMR absorptions (C=O 175.2 ppm, $CH(CO_2H)_2$ 51.8 ppm).

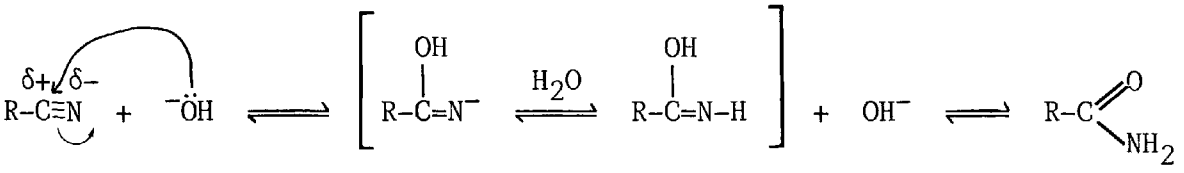
6.2 Conversion of 1-Chloroalkynes to the Acetylenic Acid via the Addition of One Carbon Atom

1-Chloroalkynes of chain lengths 11, 13, 15, 17 and 19 were converted to acetylenic acids via the nitrile and subsequent alkaline hydrolysis, without isolation in a manner similar to that employed by Ahmad[111] and other workers.[112,113]

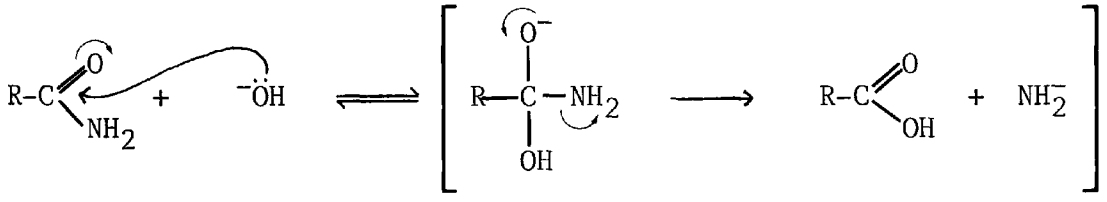
$$CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl \xrightarrow{EtOH/NaCN} CH_3(CH_2)_nC{\equiv}C(CH_2)_mC{\equiv}N$$

$$\xrightarrow{KOH} CH_3(CH_2)_nC{\equiv}C(CH_2)_mCO_2H$$

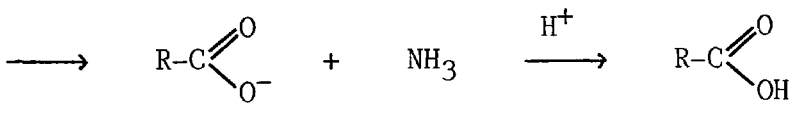
Formation of the nitrile proceeds via an S_N2 reaction. The nitrile is relatively electrophilic and upon hydrolysis undergoes nucleophilic addition of a hydroxide ion to the polar C≡N bond to form a hydroxy-imine. This is rapidly converted to an amide.



The amide readily undergoes base-catalysed hydrolysis via a nucleophilic addition/elimination reaction.



[A]



[B]

The reaction is essentially irreversible as the leaving group, NH_2^-,

removes a proton from [A] to form the stabler pair of carboxylate anion, [B], and NH_3. Loss of the latter from the hot basic solution tends to drive the reaction to completion. Subsequent acidification of this carboxylate anion yields the acetylenic acid.

Analysis of the crude reaction product indicated the presence of three main contaminants, the characterisation of which are now briefly summarised.

Alcohols and Ethers. On occasions, conversion of the 1-chloroalkyne to the nitrile was not complete. This resulted in the formation of acetylenic alcohols when sodium hydroxide was introduced to the reaction vessel to commence hydrolysis.

$$CH_3(CH_2)_nC{\equiv}C(CH_2)_mCl \xrightarrow{NaOH} CH_3(CH_2)_nC{\equiv}C(CH_2)_mOH$$

During acidification, protonation of these alcohols can occur which then react with the elimination of water to form the ether.

$$2CH_3(CH_2)_nC{\equiv}C(CH_2)_mOH \xrightarrow{H^+} [CH_3(CH_2)_nC{\equiv}C(CH_2)_m]_2O$$

If ethanol is also present during acidification, the diethyl ether and the ethyl alkyne ether may also result:-

$$2CH_3CH_2OH \xrightarrow{H^+} [CH_3CH_2]_2O$$

$$CH_3(CH_2)_nC{\equiv}C(CH_2)_mOH + CH_3CH_2OH \xrightarrow{H^+} CH_3(CH_2)_nC{\equiv}C(CH_2)_mOCH_2CH_3$$

Isolation and spectroscopic analysis of these neutral by-products indicated that, in most cases where appreciable amounts were formed, the ethyl alkyne ether predominated. The IR spectrum of the neutral

components isolated during the purification of 6-tetradecynoic acid (14:1(6)a) for example, exhibits only one outstanding absorption other than C-H stretching and bending. This is a strong, sharp absorption at 1110 cm^{-1}, a region in which the C-O stretching of ethers occurs and as such, is typical of a primary ether.

In addition to chemical shifts typical of a long chain acetylenic compound, the ^{1}H NMR spectrum of the same sample exhibits three absorptions characteristic of an ethyl alkyne ether. The chemical shifts of such compounds may be summarised as below:-

$$\underset{0.88}{CH_3}-\underset{1.27}{(CH_2)_x}-\underset{2.13}{CH_2}-C\equiv C-\underset{2.13}{CH_2}-\underset{1.27}{(CH_2)_x}-\underset{1.57}{CH_2}-\underset{3.42}{CH_2}-O-\underset{3.41}{CH_2}-\underset{1.22}{CH_3}$$

The signal at about 3.4 ppm is composed of two absorptions, attributable to methylene protons α to oxygen. The methylene protons of the ethyl group absorb as a quartet, and superimposed on this is a triplet for the C-1 methylene protons of the alkyne. The well defined triplet which results from the chemical shift of the methyl protons of the ethyl group is partially superimposed on the large absorption signal of the polymethylene protons.

Further evidence for the formation of this ether may be derived from ^{13}C NMR which exhibits typical ethereal absorptions between 62 and 72 ppm. Other minor absorptions in this region indicate the presence of other ethers in minor amounts.

Esters. The formation of ethyl alkynoates (3-7%) arose during acidification from the reaction of the resulting acetylenic acid with residual ethanol via Fischer esterification. The presence of these ethyl alkynoates may be indicated from characteristic shifts in the ^{13}C NMR

spectra (~174 ppm, C=O; ~66 and 15 ppm, $-O-CH_2CH_3$).

Esters when formed are easily removed and their presence is minimised by removing most of the ethanol prior to acidification and then careful control of the acidification procedure at 0^oC.

In addition to by-products, trace amounts of nitriles and amides were occasionally recovered as a result of incomplete hydrolysis. Their presence may be indicated by ^{13}C NMR from C≡N and $CONH_2$ chemical shifts which absorb at about 113.8 and 162-163 ppm respectively. Increasing hydrolysis time minimised the recovery of these compounds.

The synthesised acetylenic acids were purified by dissolving in aqueous alkali and extracting with ether to remove the neutral products. Subsequent acidification of the aqueous layer, and extraction with ether, yielded the acetylenic acids which were then either recrystallised from petroleum ether ($40-60^o$) at 0^oC and dried under vacuum to leave white crystals, or distilled under reduced pressure resulting in a pale yellow oil. The acidification reaction is exothermic and if uncontrolled can result in rearrangement of the product. It is essential therefore that acidification is performed slowly and at 0^oC. As a precautionary measure, the acids were stored at -20^oC under nitrogen in sealed glass screw-capped bottles until required.

Yields and melting points are summarised in Table 28. Confirmation of conversion was obtained from characteristic features in the IR (C=O(st) 1710 cm^{-1}, OH(st) 3500-2500 cm^{-1}) and ^{13}C NMR (C=O ~180 ppm) spectra. For comparative purposes, spectroscopic characteristics are discussed in detail along with other monounsaturated acids in Part Two, Section Three.

TABLE 28

Melting Points and Yields of Acetylenic Acids of General Formula $CH_3(CH_2)_nC{\equiv}C(CH_2)_mCO_2H$

Acetylenic Acid	n	m	m.p.(°C)	Literature value(°C)	Reference	Yield(g)	Yield(%)[a]
5-Dodecynoic	5	3	21.0-22.5	-	-	6.5	62.3
6-Dodecynoic	4	4	b	-	-	7.4	59.0
7-Dodecynoic	3	5	19.5-20.5	18.5-19.5	111	20.8	90.0
8-Dodecynoic	2	6	36.0-38.0	41.5-42.5	152	15.9	80.0
9-Dodecynoic	1	7	39.0-40.0	39.5-41.0	152	6.7	52.0[c]
5-Tetradecynoic	7	3	35.5-36.0	33.0-34.0	152	7.2	71.0
6-Tetradecynoic	6	4	31.0-32.0	-	-	13.4	68.2
7-Tetradecynoic	5	5	29.0-30.0	29.5-30.0	111	23.4	74.7
8-Tetradecynoic	4	6	23.0-24.0	22.0-22.5	90	20.3	77.6
9-Tetradecynoic	3	7	31.5-32.5	30.0-32.0	152	5.0	45.3[c]
10-Tetradecynoic	2	8	26.0-28.0	-	-	18.7	81.3
11-Tetradecynoic	1	9	49.5-50.5	47.0-49.0	152	13.8	77.5
5-Hexadecynoic	9	3	43.5-45.0	-	-	5.7	61.2
6-Hexadecynoic	8	4	39.0-40.0	-	-	7.3	70.2
7-Hexadecynoic	7	5	38.0-39.0	38.0-39.0	173	6.3	75.8
8-Hexadecynoic	6	6	31.5-33.0	-	-	8.3	79.7
10-Hexadecynoic	4	8	34.5-35.5	35.0-36.0	90	22.9	88.1
11-Hexadecynoic	3	9	38.8-39.5	-	-	21.7	83.4
12-Hexadecynoic	2	10	42.0-42.5	41.0-42.0	115	12.5	70.9
13-Hexadecynoic	1	11	56.0-57.5	-	-	8.3	50.1[c]
7-Octadecynoic	9	5	48.2-49.2	48.0-49.0	116	7.2	69.3
8-Octadecynoic	8	6	46.0-47.0	46.5-47.0	116	21.6	82.3
9-Octadecynoic	7	7	45.5-46.5	46.0-46.5	116	4.7	58.1
10-Octadecynoic	6	8	45.5-46.5	45.5-46.5	116	21.5	80.0
12-Octadecynoic	4	10	46.0-47.0	46.0-47.0	116	7.8	75.3
13-Octadecynoic	3	11	48.0-49.0	48.5-49.5	116	3.3	55.6[c]
14-Octadecynoic	2	12	63.0-64.0	63.5-64.0	116	3.9	54.0
9-Eicosynoic[d]	9	7	50.0-51.0	-	-	0.8	58.0[c]
10-Eicosynoic	8	8	49.0-50.0	-	-	4.5	72.7
11-Eicosynoic	7	9	49.7-50.5	-	-	6.8	64.6
12-Eicosynoic	6	10	51.0-52.0	-	-	6.5	63.0
13-Eicosynoic	5	11	52.0-54.0	-	-	1.3	54.3[c]
14-Eicosynoic	4	12	55.0-55.5	54.5-55.5	90	4.0	77.5
15-Eicosynoic	3	13	57.0-58.0	-	-	1.8	52.9[c]

FOOTNOTES

a) Based on 1-chloroalkyne unless indicated otherwise.

b) b.p. 129-130°C/0.5mm.

c) Based on the *gem*-diethyl ester of the monoalkyne.

d) C_{20} acids also called Icosynoic in some literature sources.

7 The Stereospecific Reduction of Acetylenic Acids to Alkenoic Acids

The *cis*- and *trans*-alkenoic acids were synthesised by partial reduction of the acetylenic acid. The partial reduction of acetylenic compounds has been studied by several workers and widely employed in the synthesis of olefinic compounds. Generally catalytic hydrogenation over an appropriate catalyst gives predominantly the *cis* isomer whereas chemical methods of reduction give the *trans* isomer.[174]

Generally, for long term storage, the acids were kept in the acetylenic form and reduced as required. Typically, reductions involved 1-4g of acetylenic acid, depending on availability.

7.1 The Synthesis of *cis*-Alkenoic Acids

Numerous catalytic hydrogenations of acetylenic to the corresponding *cis*-alkenoic acids have been recorded. Much of the earlier hydrogenations employed a W6 Raney nickel catalyst.[110,111,112,153] Invariably, overhydrogenation occurred resulting in most cases in a mixture of acetylenic, *cis*-alkenoic and saturated acids.

In a study of the reduction of stearolic (9-octadecynoic) acid by this method, Khan[175] found that fractional crystallisation of the crude product after 1 mole of hydrogen per mole of alkyne had been absorbed gave 72% of oleic, 16% of stearolic and 12% of stearic acids, showing that the catalyst was not completely selective. Examination of the crude product by IR spectroscopy indicated the presence of ca. 6% of *trans* alkenoic acid, indicating that the reaction was not completely stereospecific. Other authors have also commented on the formation of small amounts of *trans* and saturated acids during the partial reduction of acetylenic acids over W6 Raney nickel, although report that their

formation is of little consequence as these impurities can usually be removed.

Henne and Greenlee reported that reducing the activity of the catalyst reduces the likelihood of overhydrogenation.[107] This may be achieved by using a catalyst of the nickel type at as low a temperature and low hydrogen pressure as possible. Using nickel supported on kieselguhr, and operating at low temperature and pressure, there was little tendency to hydrogenate further than the alkene, and the absorption of hydrogen slowed almost to a stop as the amount used approached 1 mole per mole of alkyne.[107] Furthermore, interruption of the hydrogenation with the absorption of only 0.8 mole of hydrogen per mole of alkyne minimised the formation of the saturated compound.

Alternatively, the use of colloid support catalysts such as palladium have been reported in the literature.[103] Up until the 1950s however, relatively little use had been made of palladium catalysts for the partial reduction of acetylenic compounds. This unpopularity arose from the catalyst's apparent lack of stereospecificity and selectivity although partial "poisoning" of the catalyst subsequently circumvented this problem.

Early publications reported that partial hydrogenation of both stearolic and behenolic acid over 1% palladium-barium sulphate gave a mixture of *cis* and *trans* isomers of the corresponding alkenoic acid, together with starting material and the saturated acid.[176] A similar lack of stereospecificity and selectivity in the reduction of stearolic acid over 0.5% palladium-calcium carbonate has been noted by other workers[177] although this catalyst was reported by Ames and Bowman to give high yields of

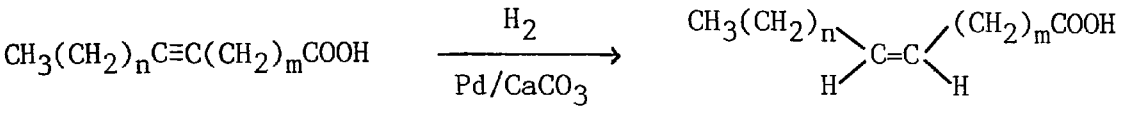
cis-9-undecenoic acid.[178] A high degree of specificity has also been reported using 5% palladium-barium sulphate in the presence of quinoline.[115,173] Other workers have recommended 5% palladium-charcoal in an alcohol-pyridine medium, claiming that much less saturated acid is formed by this procedure than by the use of W6 Raney nickel.[179]

In 1952 Lindlar developed a catalyst for the partial hydrogenation of acetylenic compounds[104,105] and this catalyst has been used with conspicuous success for the formation of *cis*-alkenoic acids. Lindlar's catalyst is palladium on calcium carbonate partially "poisoned" with lead acetate. This partial poisoning ensures that hydrogenation ceases after the alkene is formed and does not proceed to give the saturated compound.

Baker et al. have reduced stearolic acid over this catalyst in ethyl acetate with a small amount of quinoline to enhance selectivity.[177] One mole of hydrogen was rapidly absorbed and the reaction then became extremely slow. Crystallisation of the product gave oleic acid in 74% yield but no stearic or stearolic acid. Chromatographic analysis of the crude product failed to detect any significant quantities of stearolic or stearic acids although IR analysis of the methyl ester derivative indicated the presence of about 5% of elaidic acid. It was further determined that double bond migration did not occur during hydrogenation. Gunstone et al. have subsequently used this method for the synthesis of *cis*-alkenoic acids and report a minimum amount of contamination with *trans* acids.[90,117]

In view of these observations, Lindlar's catalyst was employed for the preparation of *cis* acids from the acetylenic compounds synthesised in

this study.



The preparation of Lindlar's catalyst is well documented but it is also commercially available. In view of the catalyst's reported stability and retention of activity on storage, the catalyst was obtained from Aldrich.

As catalytic hydrogenation is a heterogeneous process rather than a homogeneous one, it has proved very difficult to study for mechanistic purposes. Nevertheless, although many details remain uncertain, a rudimentary understanding of the mechanism and stereochemistry of catalytic hydrogenation has been developed.

Briefly, the atoms on the surface of a crystal body of a metal catalyst possess a "residual combining power" with which both unsaturated compounds and hydrogen react exothermically and reversibly. With acetylenic compounds (and alkenes), this probably involves its π electrons as alkanes are not similarly adsorbed. No π electrons are available in the hydrogen molecule either, and its adsorption must involve considerable weakening of its σ bond, although not necessarily complete fission to yield $H^{\bullet}$ atoms.

The actual spacings of the metal atoms in the surface are important in making one face of a metal crystal catalytically effective, and another not, depending on how closely the actual atom spacings approximate to the bond distances in the acetylenic acid and hydrogen molecules. In practice, only a relatively small proportion of the total metal surface is found to be catalytically effective; the so called "active points"

These adsorb the acetylenic acid strongly, and then desorb immediately the resultant alkene thus becoming free for further adsorption of acetylenic acid.

In view of this "lining up" of acetylenic molecules on the catalyst surface, and probable approach of activated hydrogen from the body of the metal, hydrogenation in theory proceeds stereoselectively SYN i.e. both hydrogens adding from the same side, despite the fact that this leads to the more crowded, thermodynamically less stable *cis* acid. In practice, this is broadly the case although analysis of the crude reaction products, by ^{13}C NMR and the methyl ester derivatives by GLC using an OV-275 packed column, indicated the formation of small, but varying amounts of *trans* acids. Their recovery indicated that stereoselectivity is often short of 100% SYN and can be influenced by reaction conditions, sometimes being far short of 100% SYN. Although the mechanism of hydrogenation is highly complex, it has been established that the two hydrogens are not added to the acetylenic bond simultaneously and the reason for <100% SYN stereoselectivity thus becomes apparent.

Generally, hydrogenations were performed by dissolving 1-4g of the acid (depending on availability) in an appropriate solvent and agitating the mixture over Lindlar's catalyst in an atmosphere of hydrogen. Initial runs indicated that a high degree of purity in the acetylenic acid and solvent medium was essential. Hydrogenation of unpurified acetylenic acids was very slow or more often than not, failed completely because of poisoning of the catalyst by impurities. Once purified however, hydrogen uptake was initially rapid, steadied at a constant rate, and slowed slightly before ceasing abruptly after the uptake of an equimolar

amount.

Analysis of the crude products recovered from hydrogenation of pure acids in absolute ethanol only, revealed the presence of varying amounts of *trans* and unreacted acetylenic acids. Proportions of *trans* components detected were on average about 5-7% although in some cases, recovery was as high as 14%. Acetylenic acids were present to a lesser extent, usually 0.5-2%. In an attempt to reduce *trans* acid formation, hydrogenation was performed over a range of different conditions.

The hydrogenation reaction, applied to the conversion of poly-ynoic to all *cis*-polyenoic acids using Lindlar's catalyst, has been investigated by Pabon et al..[180] They optimised reaction conditions by subjecting 10,13-nonadecadiynoic acid to hydrogenation over Lindlar's catalyst with various solvents at different temperatures. Optimum results were obtained when either ethyl acetate, light petroleum or acetone were used as solvents. The employment of ethanol, they claimed, lowered the purity of the product from 98-99% to 95-96%. Furthermore, the addition of a small amount of quinoline (1-2 ml/g catalyst) to the reaction medium was found to be beneficial. Lowering the reaction temperature only resulted in lower reaction rates.

Bearing these observations in mind, hydrogenation was performed at room temperature using the solvents employed by Pabon et al. and optimum results were obtained when either ethyl acetate or ethanol were employed. The use of light petroleum or acetone was found to be unsuitable. No significant difference was detected in the formation of *trans* components between hydrogenations in ethanol or ethyl acetate alone. In the presence of a small amount of quinoline however, the

formation of *trans* components was minimised and in some cases, was undetectable. The presence of an excess amount of quinoline, however, is detrimental and prevents hydrogenation.

The crude acids were purified when possible by crystallisation from petroleum ether (40-60°) at 0°C. With some acids, the low melting points prevented crystallisation. Such acids were alternatively purified by distillation under reduced pressure although these invariably retained a small degree of *trans* components. More often than not therefore, as residual contamination is minimal in any case, the acids were simply used without further purification after removal of solvent and catalyst. Acids were stored in CS_2, under nitrogen at -20°C in sealed glass screw-capped bottles. As an added precaution, the antioxidant BHQ at ca. 0.05% was added.

In cases where pure acids are required, purification may be achieved by elution of the methyl ester derivative on acid-washed Florisil impregnated with 20% of its weight of silver nitrate (98:2 hexane:diethyl ether).[181] All *trans* impurities consistently migrate ahead of the *cis* material and purity material may be demonstrated by standard procedures.

Conversion was confirmed from characteristic shifts in the ^1H (CH=CH 5.32 ppm, $-CH_2CH=$ 2.00 ppm) and ^{13}C (C=C 129.82 ppm) NMR spectra. Yields, boiling points and melting points where recorded are summarised in Table 29. Chromatographic and spectroscopic characteristics are discussed at length in Part Two, Section Three.

TABLE 29

Melting Points and Yields of *cis*-Alkenoic Acids of General Formula $CH_3(CH_2)_nCH=CH(CH_2)_mCO_2H$

cis-Alkenoic Acid	n	m	m.p./b.p.(°C)	Literature value(°C)	Reference	Yield(g)	Yield(%)[a,b]
5-Dodecenoic (Linderic)	5	3	c	1.0-1.3	182	ca. 1.5	-
6-Dodecenoic	4	4	c	-	-	ca. 1.0	-
7-Dodecenoic	3	5	115-117/0.5mm	-	-	0.61	30.2
8-Dodecenoic	2	6	120-122/0.5mm	-	-	0.74	36.8
9-Dodecenoic	1	7	c	-	-	ca. 1.5	-
5-Tetradecenoic (Physteric)	7	3	c	20.0	183	ca. 2.0	-
6-Tetradecenoic	6	4	131-135/0.5mm	-	-	0.98	48.7
7-Tetradecenoic	5	5	c	-	-	ca. 1.0	-
8-Tetradecenoic	4	6	c	-	-	ca. 0.7	-
9-Tetradecenoic (Myristoleic)	3	7	-	-4.5	184	d	-
10-Tetradecenoic	2	8	c	-	-	ca. 0.05	-
11-Tetradecenoic	1	9	145-148/0.5	-	-	0.94	50.1
5-Hexadecenoic	9	3	c	-	-	ca. 2.0	-
6-Hexadecenoic	8	4	c	-	-	ca. 1.5	-
7-Hexadecenoic	7	5	c	165-167/0.9mm	173	ca. 0.6	-
8-Hexadecenoic	6	6	c	-	-	ca. 0.7	-
9-Hexadecenoic (Palmitoleic)	5	7	-	-	-	d	-
10-Hexadecenoic	4	8	17.0-18.0	-	-	1.96	48.7
11-Hexadecenoic	3	9	c	-	-	ca. 0.5	-
12-Hexadecenoic	2	10	23.0-24.0	24.5-25.0	115	0.68	33.2
13-Hexadecenoic	1	11	c	-	-	ca. 0.06	-
6-Octadecenoic (Petroselinic)	10	4	-	28.0-29.0	115	d	-
7-Octadecenoic	9	5	11.0-12.0	12.0-13.0	117	0.17	8.4
8-Octadecenoic	8	6	22.0-23.0	23.0-24.0	117	0.65	32.3
9-Octadecenoic (Oleic)	7	7	11.0-12.0	10.0-11.0	117	0.73/d	28.0
10-Octadecenoic	6	8	22.0-23.0	22.5-23.5	117	0.364	17.9
11-Octadecenoic (*cis*-Vaccenic)	5	9	-	12.5-13.5	117	d	-
12-Octadecenoic	4	10	27.0-28.0	27.0-28.0	117	0.72	35.6
13-Octadecenoic	3	11	-	26.5-27.0	117	ca. 0.05	-
14-Octadecenoic	2	12	39.0-41.0	41.5-42.5	117	0.32	22.3
9-Eicosenoic[e] (Gadoleic)	9	7	23.0-24.0	23.0-23.5	184	0.08	45.6
10-Eicosenoic	8	8	29.0-30.0	-	-	0.11	11.4
11-Eicosenoic (Gondoic)	7	9	-	23.0-24.0	185	d	-
12-Eicosenoic	6	10	30.0-32.0	-	-	0.42	53.6
13-Eicosenoic	5	11	24.0-25.0	-	-	0.35	46.2
14-Eicosenoic	4	12	43.0-44.0	42.5	90	0.35	16.4
15-Eicosenoic	3	13	c	-	-	ca. 0.05	-

FOOTNOTES

a) Based on acetylenic acid.

b) % Yield quoted only if isolated by distillation or crystallisation.

c) Not isolated as acid. Used as prepared or further purified by argentation chromatography of the methyl ester

d) Commercially available.

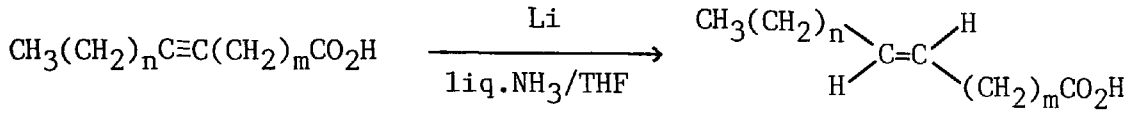
e) C_{20} acids also called Icosenoic in some literature sources.

7.2 The Synthesis of *trans*-Alkenoic Acids

The preparation of *trans* acids has previously been undertaken by stereomutation of the corresponding *cis*-isomer.[64,65,66,69,112] The synthesis exploits the equilibrium that exists under certain conditions between the *cis* and *trans* isomers of which the *trans* content is 75-80%. The isomerisation reaction is now out of favour principally because it is known to be accompanied by extensive double bond migration. Furthermore, after isolation from the reaction product, *trans* acids are recovered in insufficient amounts for extensive study.[69]

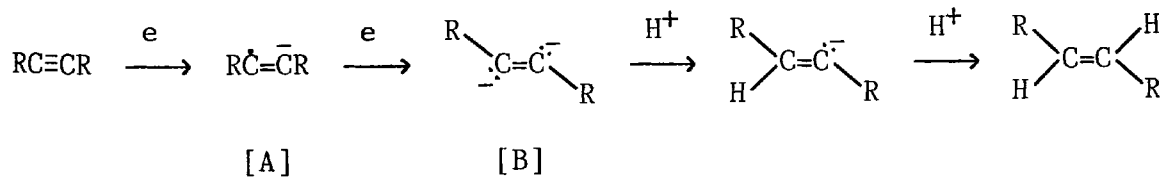
In view of these observations, and the attempt at stereomutation using selenium in this study, the method was considered unsuitable for the preparation of *trans* acids. Attention was therefore focused on the chemical reduction of acetylenic acids to *trans* alkenoic acids. The reduction of acetylenic to *trans* alkenoic compounds has previously been attempted with several reagents including sodium in methanol, and zinc dust and acetic acid.[174] No satisfactory method existed, however, until Campbell and Eby showed that acetylenes were reduced exclusively to, and only so far as, *trans* isomers by sodium in liquid ammonia.[106] Similar success was reported by Henne and Greenlee[107] who used ether as a co-solvent. Although other workers have reported these methods to be ineffective,[186] a modification however, whereby the reaction was carried out under pressure proved successful.

The method has since been employed by a number of workers although most have reported some difficulty. In this study however, acetylenic acids were satisfactorily reduced to the *trans* alkenoic acids by reaction with lithium or sodium in liquid ammonia, in the presence of THF as a co-solvent.



The method employed was basically as follows. The acetylenic acid (1-5g depending on availability), dissolved in sodium-dried THF[159] was placed in a glass-lined autoclave and distilled liquid ammonia carefully added. Lithium or sodium was then added with stirring in small pieces. After about four hours, the original volume of the reaction vessel was restored by the addition of fresh ammonia and the autoclave closed overnight during which time the temperature rose to room temperature, and the pressure increased to about 10 atm. On opening the autoclave, excess metal was destroyed by addition of solid ammonium chloride. After the addition of water and acidification, the mixture was worked-up to yield the crude acid. Crystallisation from petroleum ether (40-60°) at 0°C yielded the pure *trans* alkenoic acid.

Until recently, the mechanism for this reaction, and the consequential stereochemical course, was thought to be attributable to the addition of two electrons to the linear acetylenic bond to form a nonlinear dianion intermediate. This adopts the *trans* geometry [B] to minimise electrostatic repulsion between the two unshared electron pairs.[187] The successive addition of two protons at a faster rate than the relatively slow rate of inversion of the vinyl anion, would then account for the formation of the *trans* isomer, and much less *cis* isomer than would be expected in an equilibrium mixture.

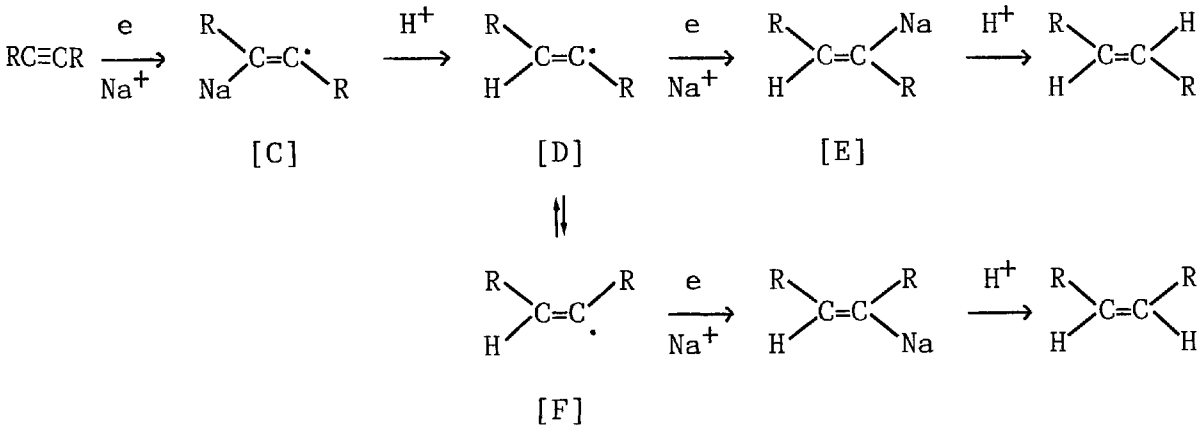


[A] [B]

This process, however, involving two successive electron transfers to the acetylenic bond to form the intermediate radical anion [A], and the dianion [B], was difficult to reconcile with polarographic studies of the electrochemical reduction of acetylenes.

It has been reported that the reduction potential for acetylene is ca. -3.0V and that alkyl substituted acetylenes accept an electron (to form [A]) only at potentials more negative than -3.0V.[188] Consequently, the reducing power of sodium in liquid ammonia (ca. -2.3V), is barely adequate to reduce such acetylenic compounds to the corresponding free radical anions [A], and is certainly inadequate to produce the corresponding free dianions [B].

House and Kinloch have therefore proposed a mechanism in which the acetylenic bond is converted successively to a *trans* sodiovinyl radical [C] (or the equivalent nonlinear radical), followed by protonation to give the *trans* vinyl radical [D].[188]

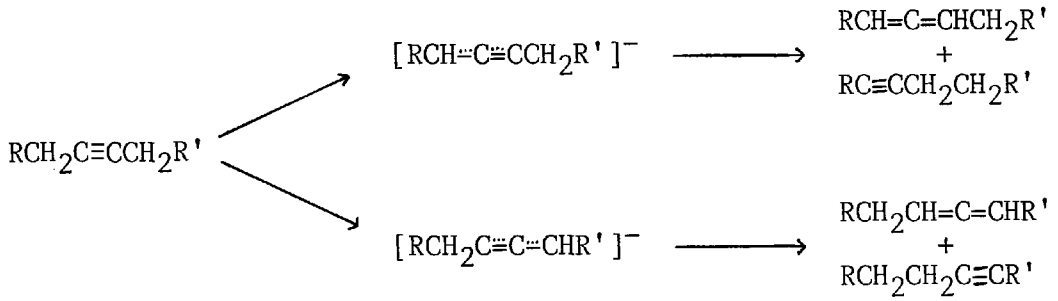


Under the conditions employed, i.e. low temperature and excess sodium/lithium, the conversion of the *trans* radical [D] to the vinyl sodium intermediate [E], is apparently slightly faster than the conversion of the *trans* radical [D] to the *cis* radical [F]. Protonation

therefore, yields predominantly the *trans* alkene.

Analysis of the crude products revealed that the predominant fraction was the desired *trans* alkenoic acid (80-95 %) and that although no *cis* acids were detected, small amounts of positional *trans* acids were formed on occasions.

The formation of these *trans* positional isomers are thought to arise from the base-catalysed isomerisation of the acetylenic bond to the allene and other acetylenes. This reaction occurs in competition with the reduction process resulting in the formation of an anion.



The resulting isomeric acetylenic acids may then be reduced in the normal manner, or isomerised further (followed by reduction) resulting in the formation of positional isomers. The formation of these isomers appeared to be promoted when sodium was used in preference to lithium as the reducing agent although their percentage formation remained well within acceptable limits.

Lithium is not as soluble as sodium in liquid ammonia and vigorous stirring was required to initiate the reaction. On several occasions, a mixture of lithium with a small amount of sodium was employed for the reduction and in such cases, the reaction proceeded satisfactorily.

In initial runs, where the reaction was performed at normal pressure,

significant amounts of unreacted material were recovered along with the *trans* acids. The recovery of acetylenic material when the reaction was performed in an autoclave, however, was negligible.

In accordance with the recommendation of Barve and Gunstone,[116] it was essential to avoid all traces of iron in the reaction mixture. Iron is a pro-oxidant that could possibly promote autoxidation of the double bond. Consequently therefore, the reaction was performed in a glass-lined autoclave, and commercial ammonia, which can contain traces of iron, distilled and filtered through glass wool prior to use. Throughout, usual procedures, as described previously for reactions involving liquid ammonia, were employed to exclude moisture from the reaction vessel.

The crude acids once formed were purified by crystallisation from petroleum ether (40-60°) or (particularly in the case of some of the shorter chain acids), simply used without further isolation. Yields and melting points where recorded are summarised in Table 30. As with the *cis* acids, *trans* acids were stored in CS_2, under nitrogen at -20°C in sealed glass screw-capped bottles, in the presence of ca. 0.05% BHQ. Conversion was confirmed from characteristic absorptions in the IR (CH=CH *trans* bend 967 cm^{-1}), ^1H (CH=CH 5.36 ppm, $CH_2CH=$ 1.95 ppm) and ^{13}C (C=C 130.32 ppm) NMR spectra. Spectroscopic and chromatographic characteristics are discussed at length in Part Two, Section Three.

8 Conversion of *cis*- and *trans*-Alkenoic Acids to the Methyl Esters

As a result of the von-volatile and highly polar nature of fatty acids, it was necessary on occasions, principally for chromatographic analysis and purification procedures, to prepare the comparatively volatile methyl ester derivatives. The esterification of fatty acids has been

TABLE 30

Melting Points and Yields of *trans*-Alkenoic Acids of General Formula $CH_3(CH_2)_nCH{=}CH(CH_2)_mCO_2H$

trans-Alkenoic Acid	n	m	m.p.(°C)	Literature value(°C)	Reference	Yield(g)	Yield(%)[a,b]
5-Dodecenoic	5	3	c	-	-	ca. 2.0	-
6-Dodecenoic	4	4	c	-	-	ca. 1.5	-
7-Dodecenoic	3	5	c	-	-	ca. 3.5	-
8-Dodecenoic	2	6	c	-	-	ca. 3.1	-
9-Dodecenoic	1	7	c	-	-	ca. 1.1	-
5-Tetradecenoic	7	3	c	-	-	ca. 1.4	-
6-Tetradecenoic	6	4	c	-	-	ca. 3.1	-
7-Tetradecenoic	5	5	c	-	-	ca. 0.7	-
8-Tetradecenoic	4	6	25.0-26.0	-	-	3.10	47.3
9-Tetradecenoic	3	7	17.0-18.0	18.0-18.5	184	2.89	44.1
10-Tetradecenoic	2	8	26.0-27.0	-	-	2.86	45.7
11-Tetradecenoic	1	9	c	-	-	ca. 3.5	-
5-Hexadecenoic	9	3	c	-	-	ca. 0.5	-
6-Hexadecenoic	8	4	32.0-33.0	-	-	1.01	34.1
7-Hexadecenoic	7	5	28.0-29.0	-	-	1.12	37.1
8-Hexadecenoic	6	6	38.0-40.0	-	-	1.91	63.2
9-Hexadecenoic (Palmitelaidic)	5	7	-	32.0-33.0	184	d	-
10-Hexadecenoic	4	8	38.0-40.5	-	-	2.47	68.4
11-Hexadecenoic	3	9	30.0-31.0	-	-	3.98	70.6
12-Hexadecenoic	2	10	45.0-46.0	-	-	2.62	44.5
13-Hexadecenoic	1	11	c	-	-	ca. 0.07	-
6-Octadecenoic (Petroselaidic)	10	4	-	52.5-53.0	116	d	-
7-Octadecenoic	9	5	43.0-44.5	43.5-44.5	116	2.89	71.7
8-Octadecenoic	8	6	50.0-52.0	51.0-51.5	116	2.16	53.6
9-Octadecenoic (Elaidic)	7	7	43.0-45.0	43.5-44.5	116	0.45/d	22.3
10-Octadecenoic	6	8	51.0-52.0	52.0-52.5	116	2.03	50.4
11-Octadecenoic (*trans*-Vaccenic)	5	9	-	43.0-43.5	116	d	-
12-Octadecenoic	4	10	52.0-53.0	52.0-53.0	116	0.53	26.6
13-Octadecenoic	3	11	44.0-45.0	43.5-44.5	116	0.86	56.9
14-Octadecenoic	2	12	54.0-55.0	53.0-53.5	116	0.92	60.9
9-Eicosenoic[e] (Gadelaidic)	9	7	54.0-55.0	54.0	184	0.04	35.8
10-Eicosenoic	8	8	51.3-53.0	-	-	1.00	49.7
11-Eicosenoic	7	9	40-0-41.0	-	-	1.93	47.9
12-Eicosenoic	6	10	57.0-58.5	-	-	1.09	43.3
13-Eicosenoic	5	11	c	-	-	ca. 0.05	-
14-Eicosenoic	4	12	59.0-60.0	-	-	1.23	61.1
15-Eicosenoic	3	13	c	-	-	ca. 0.07	-

FOOTNOTES

a) Based on Acetylenic Acid.

b) % Yield quoted only if isolated by distillation or recrystallisation.

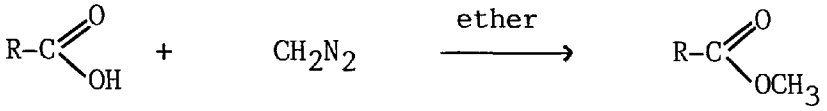
c) Not isolated as acid. Used as prepared or further purified as necessary by argentation chromatography.

d) Commercially available.

e) C_{20} acids also called Icosenoic in some literature sources.

comprehensively reviewed.[189,190,191] Basically, the conversion of acids to their methyl esters may be undertaken by one of two general methods.

Conversion into methyl esters may be effected by the rapid reaction at room temperature of the fatty acid with diazomethane in the presence of a little methanol which catalyses the reaction.



The diazomethane method of ester synthesis is ideal since it occurs cleanly, under mild conditions, and gives nitrogen as the only by-product. As such, it has been used with conspicuous success in the preparation of fatty acid methyl esters.[192] As a result of the hazardous nature of diazomethane and precursors involved in its synthesis however,[193] it is recommended that it should only be used when no other reagent is suitable.[191]

The esterification of synthesised acids and acids derived from dietary lipids can be readily achieved by heating with a large excess of anhydrous methanol in the presence of a mineral acid catalyst. Suitable reagents which have been used for this purpose include a solution of 1-2% (v/v) concentrated sulphuric acid in methanol, boron trifluoride in methanol (12-14% w/v)[194] and boron trichloride in methanol.[195]

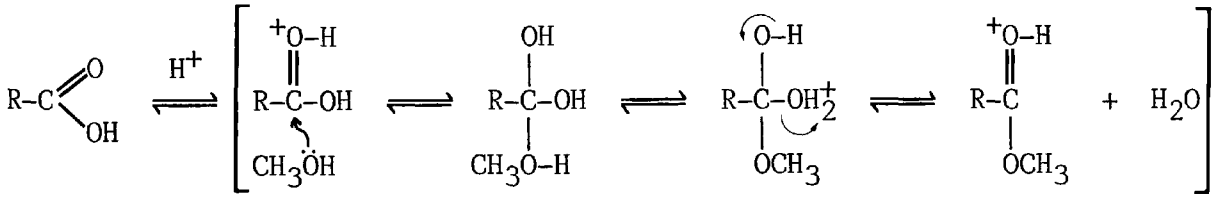
The commonest and mildest reagent available for the purpose and the method employed in this study is 5% (w/v) anhydrous hydrogen chloride in methanol as this is considered by Christie to be less likely to lead to side reactions than the use of other reagents.[191] The anhydrous methanolic hydrogen chloride was conveniently prepared by the slow addition of freshly distilled acetyl chloride to cooled ($0^{o}C$), anhydrous

methanol.[196] Methyl acetate is formed as a by-product but does not interfere seriously with the esterification.

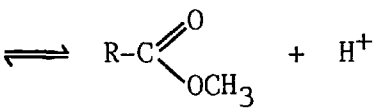
$$CH_3OH + CH_3COCl \longrightarrow CH_3CO_2CH_3 + HCl$$

The procedure is very satisfactory provided the temperature is not allowed to rise above 0°C and the reagent is prepared fresh immediately before use. Esterification is an equilibrium reaction and the presence of water tends to favour the formation of the acid or at least prevents the reaction going to completion. Accordingly therefore, anhydrous methanol was prepared in accordance with the procedure described by Vogel for super dry ethanol and stored over Type 4A molecular sieve prior to use.[172]

Conversion was effected by heating anhydrous methanolic hydrogen chloride and acid in pentane in a sealed tube at 60°C for 2-3 hours. The method, although initially developed for the esterification of small amounts of lipids, may be scaled up considerably. The reaction proceeds via the Fischer esterification mechanism, also referred to as $A_{AC}2$ (acid-catalysed, acyl-oxygen cleavage, bimolecular), and within the limits of experimental error, conversion is 100%. The methyl esters so formed were used without any further purification and the spectroscopic and chromatographic properties thereof characterised.



$R=CH_3(CH_2)_nCH=CH(CH_2)_m$
c/t



SECTION THREE

THE CHARACTERISATION OF LONG CHAIN MONOUNSATURATED FATTY ACIDS

In addition to the synthetic work, the qualitative characterisation of these positional and geometrical isomers of monounsaturated fatty acids by chromatographic and spectroscopic methods was undertaken.

The analysis of fatty acids by various chromatographic and spectroscopic techniques has been comprehensively reviewed.[197,198,199] Traditionally, the determination of *trans* unsaturated fatty acids has been carried out by IR spectroscopy.[53,200] Packed column GLC with very polar stationary phases (e.g. Silar 10C, SP-2340 or OV-275), has been used for the quantitative determination of the *cis* and *trans* percentages of monounsaturated FAMEs[57,58] as have modes of argentation chromatography.[199] The quantification of positional isomers of fatty acids has, however, proved more of a problem. Capillary columns, coated with the phases mentioned above, have resolved positional and geometrical isomers of monounsaturated FAMEs to some extent, however some of the *cis* isomers overlapped with the *trans* isomers.[198]

In most common techniques used for the analysis of positional isomers, the monounsaturated acids are first separated as the methyl esters by preparative GLC. These are then separated into their *cis* and *trans* forms by argentation TLC and analysis of the ozonide cleavage products by GLC.

This technique has been used with relative success to determine the content of isomeric monounsaturated fatty acids in bovine milk fat,[54] HVO and HMO, and the degree of incorporation into organ lipids after being fed HF.[32,201] It gives almost complete information on all isomeric monounsaturated fatty acids but consists of many steps and is therefore time consuming and laborious. An alternative, rapid technique is therefore desirable for the analysis of the large number of samples

necessary in a case vs. control study.

With recent improvements in instrumentation, the determination of double bond position by MS and NMR spectroscopy have become increasingly popular. The mass spectra of appropriate derivatives of fatty acids have been reported but these techniques have not been applied to the analysis of complex fatty acid mixtures.[198] By comparison, NMR spectroscopy, which exhibits characteristic chemical shifts for various fatty acids, has been applied to the analysis of fatty acids in oils and fats.[198,202,203,204]

The techniques employed in this investigation were principally packed and capillary column GLC, reverse-phase HPLC on C_{18}-bonded phases, argentation column chromatography, IR and NMR spectroscopy.

Argentation column chromatography was used to purify the methyl esters of synthesised acids when necessary. Geometrical isomers in this mode of chromatography exhibit different retention characteristics.

As a result of the continuous nature of the synthetic work and instrument limitations, the analysis of monounsaturated fatty acids by chromatographic techniques (particularly capillary column GLC) was limited to commercially available acids, some lipid mixtures and synthetic acids as they became available. Nevertheless, the limited amount of data obtained allowed several generalisations to be made regarding the separations of positional isomers by GLC and HPLC.

In contrast to chromatographic methods, all acids were characterised by ^{1}H and ^{13}C NMR spectroscopy. ^{13}C in particular was indicative of geometrical and positional isomerism. In addition, the ^{17}O spectra of

some acids were also recorded. For comparative purposes, the spectra of acetylenic, saturated and polyunsaturated acids are also discussed.

9 Chromatographic Characterisation

9.1 Argentation Column Chromatography

In argentation chromatography, the property exhibited by silver compounds of forming polar complexes reversibly with double bonds is used as a means of separating fatty acids according to the number and configuration of those double bonds. The first procedure in which the principle was applied was a countercurrent distribution system, but it is now more generally used in conjunction with adsorption (thin layer and column) chromatography. The principle and applications of the method have been reviewed.[199,205] Analytical separations have been achieved by HPLC on columns of silica impregnated with silver nitrate.[206] The separation of positional isomers by argentation TLC has also been reported.[207]

In this investigation, synthesised acids were purified as the methyl esters when required by elution on acid-washed Florisil (Sigma) impregnated with 20% of its weight of silver nitrate (98:2 hexane:diethyl ether).[181] Here, *trans* acids consistently migrate ahead of *cis* material and purity was demonstrated by GLC (Part Two, Section Three, 9.2).

Columns were protected from light by wrapping with aluminium foil. Furthermore, as it has been reported that silver complexes are more stable at lower temperatures,[197] columns were cooled with a water jacket to enhance separations.

9.2 Gas Liquid Chromatography - Packed and Capillary

The vast explosion of information on the chemistry and biochemistry of lipids over the past 26 years has been principally because of the development and exploitation of GLC. The method and its application has been reviewed by Christie.[197]

Geometrical isomers of unsaturated fatty acids are not, in general, separable on conventional polyester liquid phases such as EGSS-X and EGSS-Y but some excellent separations have been recorded with the newer cyanoalkylpolysiloxane phases such as Silar 10C, SP-2340 or OV-275.[197]

In this study, the separation of geometrical and positional isomers of FAMEs was evaluated on both packed and capillary columns. The packed column was a 40' x 1/8" stainless steel column packed with 20% OV-275 coated on 100/120 mesh acid-washed and silanised Chromosorb P. The capillary column was a 50m x 0.25mm fused silica open tubular column coated with Silar 10C, film thickness 0.2 microns. In both cases, the carrier gas was nitrogen and the instrument was a Perkin-Elmer F-17 gas chromatograph fitted with dual flame ionisation detectors (FID), and interfaced with a Perkin-Elmer 3600 data station. For capillary column GLC, the gas chromatograph was fitted with an injection splitter set at ratio 1:40.

Figures 20 and 21 illustrate typical chromatograms of adipose tissue FAMEs on packed OV-275 and capillary Silar 10C coated columns respectively. Operating conditions were as follows: i) OV-275, column temperature 220°C, flow rate 30 ml/min and optimum ester loading 2µl of CS_2 solution (ca.13 mg/ml). ii) Silar-10C, injector and detector temperature 220°C, column temperature isothermal at 100°C for 10 minutes,

FIGURE 20 GLC Separation of Adipose Tissue Fatty Acid Methyl Esters on an OV-275 Coated Packed Column

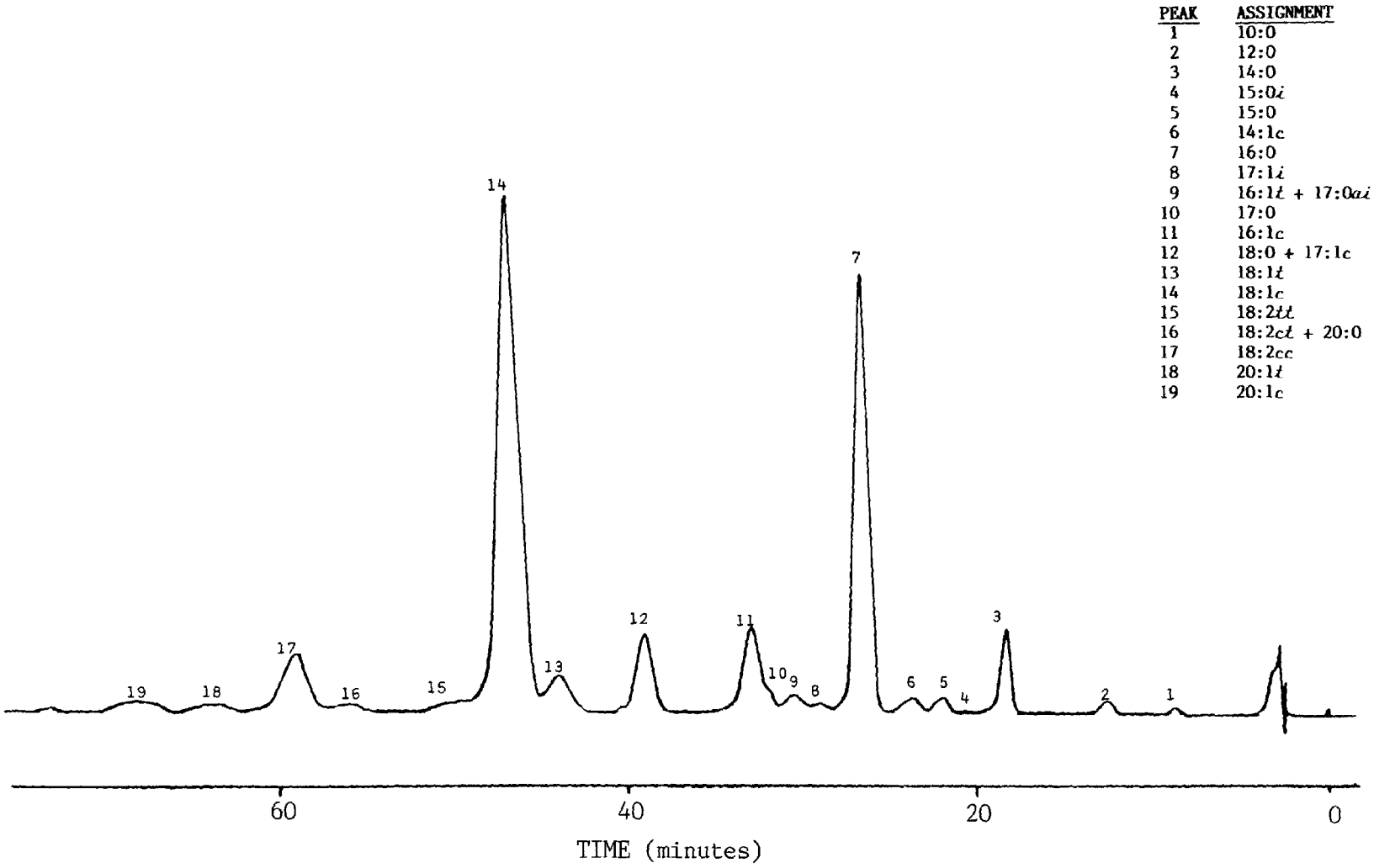
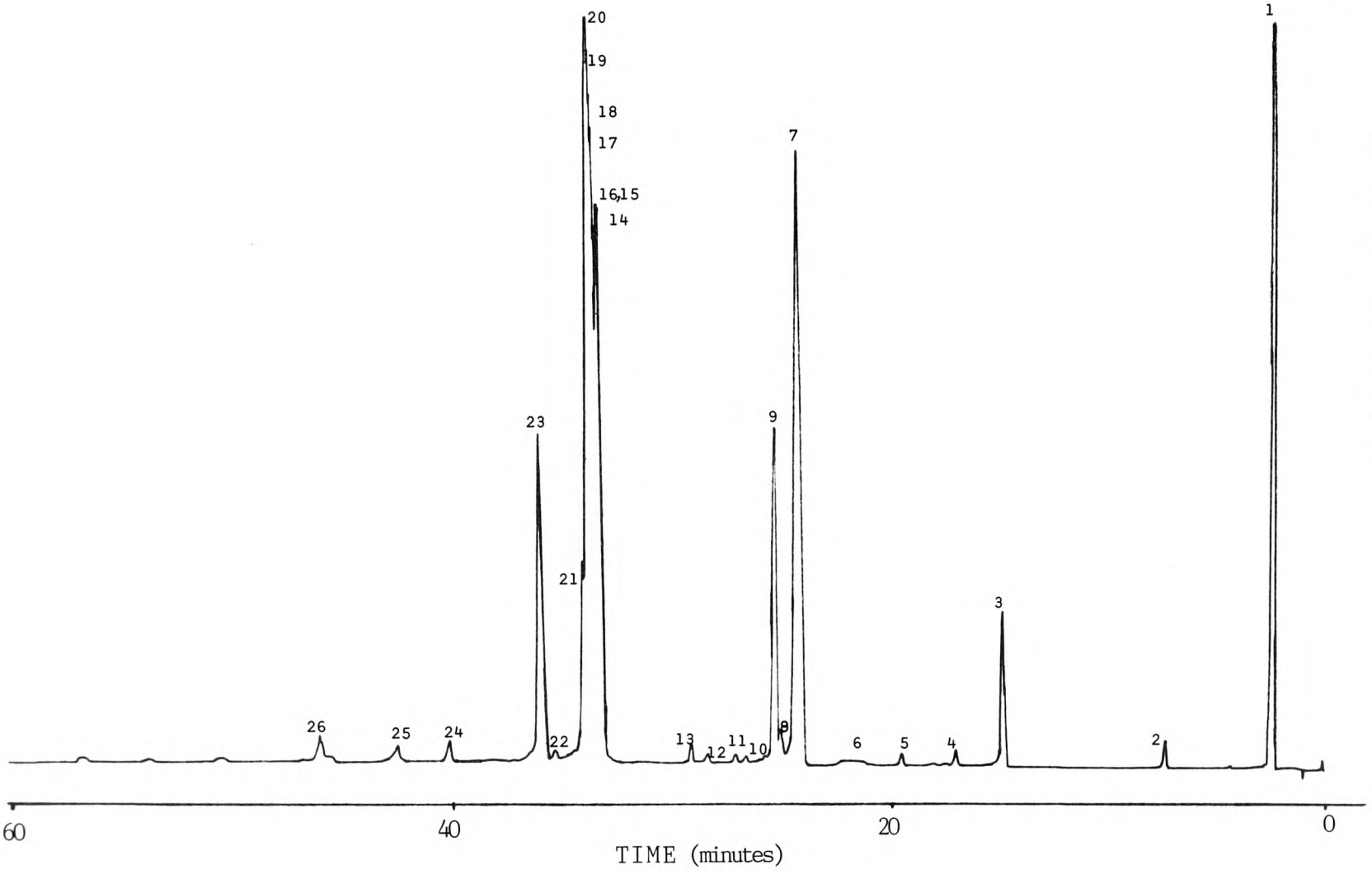


FIGURE 21 GLC Separation of Adipose Tissue Fatty Acid Methyl Esters on a Silar 10C Coated Fused Silica Capillary Column



Peak	Assignment
1	10:0 + CS_2
2	12:0
3	14:0
4	15:0*i*
5	15:0
6	14:1
7	16:0
8	16:1*t*
9	16:1*c*
10	17:0*i*
11	17:0*ai*
12	17:0
13	17:1*c*
14	18:0
15	Geometrical and Positional Isomers of 18:1
16	
17	
18	
19	
20	
21	
22	18:2*tt*
23	18:2*cc*
24	20:0
25	20:1*t*
26	20:1*c*

thereafter linearly programmed at 2°/min until 140°C, flow rate 3 ml/min, and optimum ester loading of 0.5µl of CS_2 solution (ca.13 mg/ml).

Figure 20, demonstrates that although isomers are separable on the basis of geometry by packed column GLC, some acids co-elute. Thus, whereas 18:1 *trans* is directly quantifiable, 16:1 *trans* is composite with 17:0 *anteiso*. The separation of geometrical isomers in a complex mixture is not baseline as in the separation of a standard mixture of 18:1(9)c and 18:1(9)t (Figure 24). Analysis of a standard mixture containing geometrical and positional isomers of monounsaturated FAMEs also resulted in non-baseline separation. The non-baseline separation of geometrical isomers in Figure 20 is probably due to positional isomerism.

Although this mode of chromatography did not resolve positional isomers, it has been established as a routine method for the quantification of geometrical isomers in dietary and human tissue lipids.[57,58,59] It was used extensively in this study to determine the purity of the synthesised acids.

Despite instrumentation limitations, the results obtained with capillary column GLC, in view of literature data,[198,201,208,209] were disappointing. Conditions for separation in Figure 21 were not optimised however, and it is possible that resolution could be further improved. Encouragingly, simple mixtures of positional and geometrical isomers were partially resolved although these did not compare with other reported separations.

Nevertheless, it is possible to make the following general characterisations regarding the separation of geometrical and positional fatty

acid isomers by GLC. Retention increases with increasing chain length. The retention of *trans* acids are intermediate between those of *cis* and saturated acids. Retention increases with increasing unsaturation, thus 18:0 elutes before 18:1 etc. Retention increases with decreasing distance of the position of unsaturation from the carbonyl end of the chain.

In spite of the disappointing results obtained here, it is considered that development of this technique offers the best prospects for the direct quantification of positional isomers in a complex mixture on a relatively rapid basis. Although spectroscopic techniques such as NMR proved invaluable as far as characterisation of individual acids, or the profiling of simple lipid mixtures are concerned, the quantification of complex mixtures proved more problematical.

Analysis of geometrical and positional isomers by capillary column GLC is limited by the currently available stationary phases. At the present time, it is not possible for example, to separate all the *cis* and *trans* 18:1 positional isomers. The use of columns coated with the highly polar SP-2560 liquid phase however, recently developed by Supelco (Figure 22), hold particular promise.[209]

It can be seen from Figure 22, that some overlap of geometrical isomers occurs and the initial separation of acids on the basis of chain length and degree of unsaturation may be beneficial. Svensson et al. have achieved some success in this context by combining reverse-phase HPLC and capillary column GLC.[201] Simplex procedures[210] could then be applied to optimise the separation of the monoene fractions. A disadvantage to the use of such columns are however that capillary columns in general

FIGURE 22 GLC Separation of Geometrical and Positional Isomers of Fatty Acid Methyl Esters on an SP-2560 Fused Silica Capillary Column (from Reference 209)

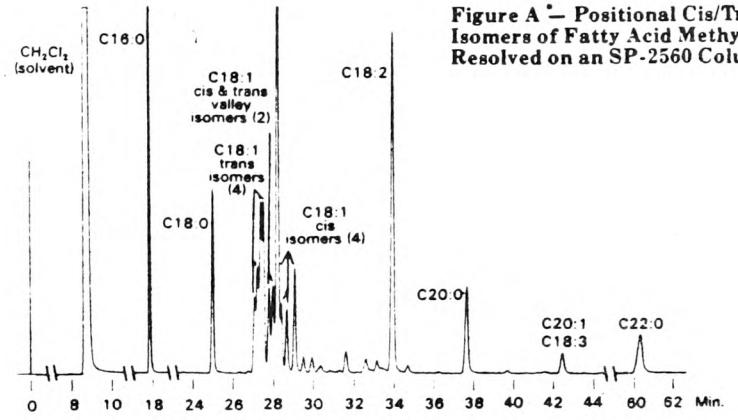


Figure A — Positional Cis/Trans Isomers of Fatty Acid Methyl Esters Resolved on an SP-2560 Column

SP-2560 fused silica capillary column, 100m x 0.25mm ID, Film Thickness: 0.20μm, Col. Temp.: 175°C, Inj. & Det. Temp.: 200°C, Linear Velocity: 20cm/sec., He, set at 175°C, Det.: FID, Sens.: 1 x 10^{-11} AFS, Sample: 1μl of Positional Cis/Trans Standard (Cat. No. 4-5170), 5.0mg/ml (overall) fatty acid methyl ester isomers in methylene chloride, Split Ratio: 100:1.

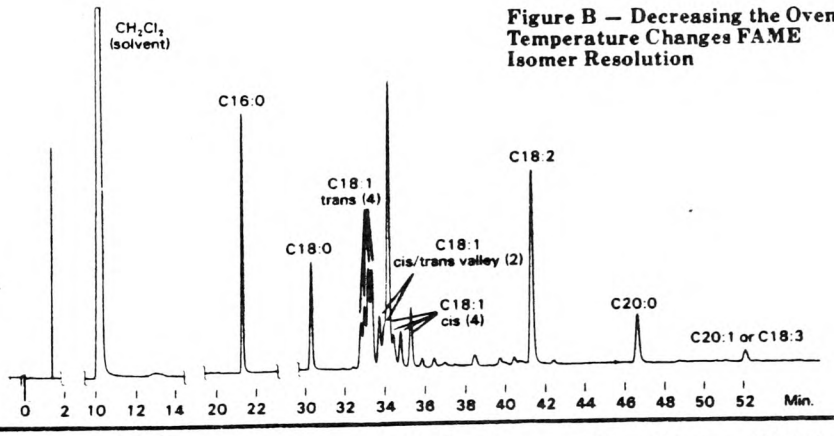


Figure B — Decreasing the Oven Temperature Changes FAME Isomer Resolution

SP-2560 fused silica capillary column, 100m x 0.25mm ID, Film Thickness: 0.20μm, Col. Temp.: 170°C, Inj. & Det. Temp.: 200°C, Linear Velocity: 20cm/sec., He, set at 175°C, Det.: FID, Sens.: 1 x 10^{-11} AFS, Sample: 1μl of Positional Cis/Trans Standard (Cat. No. 4-5170), 5.0mg/ml (overall) fatty acid methyl ester isomers in methylene chloride, Split Ratio: 100:1.

are expensive, and have a short working life compared to packed columns.

Most publications which do report separation of positional isomers do not assign specific isomers to specific peaks. Thus, the chromatographic analysis of isomeric fatty acids has been severely hampered by the lack of pure standards for most of the isomers.

With the availability of a comprehensive range of positional and geometric isomeric fatty acids as standards, and continuing developments and improvements of stationary phases, employment of capillary column GLC in future will probably provide most information regarding the direct quantification of positional isomeric fatty acids.

9.3 High Performance Liquid Chromatography

Since its inception in the mid 1960s, HPLC has been applied to effect excellent separations of fatty acids and derivatives both on an analytical and semimicro-preparative scale. The applications of HPLC to lipid analysis have been comprehensively reviewed by Aitzetmuller.[211]

Although the technique does not approach the high separation efficiency of capillary column GLC, it does offer several advantages for the separation of fatty acids. Firstly, a combination of a variety of stationary and mobile phases provides a unique selectivity which is often difficult to match even with GLC. Secondly, HPLC can provide non-destructive detection and a very good fractionation technique for subsequent analysis. In particular, HPLC may be used as an alternative to GLC on a preparative basis for the isolation of unsaturated fatty acids for subsequent analysis, as double bond isomerisation and migration, caused by the high temperatures required with GLC, may be a problem. In the same manner, HPLC may be applied to the analysis of

fatty acids that decompose at temperatures used for GLC.[212]

a) Some Practical Considerations

i) Selection of a Suitable Detector

The main limitation with the application of HPLC to lipid analysis has been the selection of a suitable detector for monitoring the separations. Differential refractometers have been employed by several workers.[201,213,214] However, as a general LC detector, it has the limitation of lack of sensitivity, lack of suitability for gradient elution and a requirement of strict temperature control. Detectors in which the eluate from HPLC columns is coated onto a moving wire which is dried to remove solvent before passing through an FID for quantification have been described but were not commercially viable.[211] Several workers have reported the use of MS to analyse fractions of fatty acid derivatives collected by HPLC separation.[215,216,217]

The most widely exploited detectors however, have been UV detectors. The original detectors were single or dual wavelength detectors which operated at 254 nm and/or 280 nm but these were subsequently followed by the introduction of variable wavelength detectors. Consequently, many of the applications of HPLC to fatty acid analysis reported in the literature are concerned with fatty acid derivatives containing strongly UV absorbing substituents on the alcohol moiety. Such derivatives have taken the form of p-methoxyanilides,[218] benzyl,[215] 2-naphthacyl,[216] p-bromophenacyl,[212] phenacyl[219,220] and methoxyphenacyl[221] esters.

Alternatively, as derivatisation is usually time-consuming and can require several steps prior to injection, the use of HPLC coupled to a variable wavelength detector operating at 205-210 nm has been proposed

as a mode of detection.[206,222] The analysis of acids and methyl ester derivatives has been reported using this technique. The method has not been greatly exploited however, because of problems with early instrumentation in working close to the detector's limit.

However, with recent improvements, and on the basis of data available, it was considered that this mode of detection would be suitable for the monitoring of fatty acids or the methyl esters on a qualitative basis in this investigation.

ii) Selection of Stationary and Mobile Phases

The separation of fatty acids by HPLC has been reported on a number of phases including silicic acid impregnated with silver nitrate.[223] Using such columns, the separation of geometrical and positional isomers of methyl octadecenoates have been reported.[206]

Most separations of fatty acids and derivatives however, have been undertaken by reverse-phase chromatography generally on columns containing C_{18}-bonded stationary phases.

In this mode of chromatography, the solutes are retained by the hydrophobic (or generally less polar) stationary phase in order of decreasing polarity. However, for columns with bonded long chain silica polymers, a mixed retention mechanism has been suggested.[224] In addition to hydrophobic phase "solubility effects," some residual adsorption and modifying effects of the mobile phase on the properties of the stationary phase (i.e. the production of a binary phase through the entrapment of solvent molecules on surface structures), are all likely to occur but in general, the exact mechanism of retention in reverse-phase columns remains a subject of much research.[225,226] The selection of the

stationary phase will be returned to in due course. Firstly however, it is necessary to consider the selection of the mobile phase.

There are several difficulties inherent with detection at 210 nm most of which are associated with the composition and the quality of the mobile phase. Appropriate precautions must therefore be taken to circumvent these.

In general, the choice of mobile phase is limited as most solvents popular as mobile phases in HPLC have a UV cut-off point above 210 nm. The most commonly used solvent pairs in reverse-phase chromatography are water/methanol and water/acetonitrile, both of which have been used in the separation of fatty acid derivatives. In addition, THF has enjoyed success as a solvent in the analysis of fatty acids and other lipids.[206,213]

However, HPLC-grade THF has a UV cut-off between 210 nm and 215 nm, and HPLC-grade methanol a maximum absorbance of 0.5 at 210 nm. Thus, extensive adjustment of the coarse zero is necessary to obtain a chromatogram and the sensitivity is greatly impaired when using these solvents.

Effectively therefore, the choice of mobile phase at 210 nm is restricted to water/acetonitrile. Commercial HPLC-grade acetonitrile has a maximum absorbance at 210 nm of 0.02-0.07. In practice however, it was found that this specification was rarely met and varied not only from supplier to supplier but also from batch to batch. Frequently further purification was necessary before the solvent was suitable for use.

As other compounds in addition to fatty acids absorb at 210 nm, a high

degree of purity is also required of the water used in the mobile phase. Polar impurities may effect column stability or elution volumes. They may accumulate at the head of the column, contaminate it and change its adsorption characteristics. Furthermore, if the solvent strength is increased as in gradient elution, they may give rise to spurious peaks in the chromatogram. HPLC-grade water was therefore prepared by double distilling deionised water before further purification using the Norganic^{TM} Water Purification System (supplied by Waters Associates/ Millipore). This procedure ensures that the water is not only particle-free, but organic free to a level of less than 0.002 absorbance units at 210 nm.

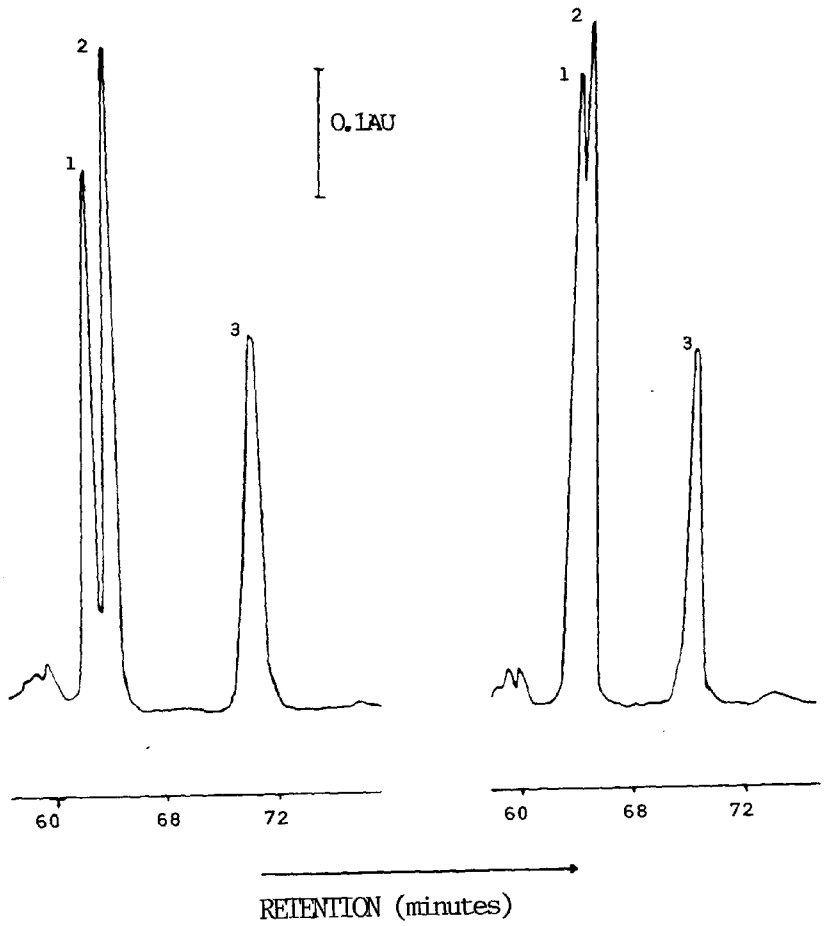
In addition, all samples were filtered using a Sample Clarification Kit (Waters Associates/Millipore) to remove any particulate matter prior to injection onto the HPLC column.

A further disadvantage of UV detection in HPLC is that in gradient work, changes in absorbance and optical effects in the sample cell can cause baseline variation in the chromatogram. However, running a blank gradient prior to analysis circumvents such problems.

Returning to the selection of the stationary phase, evaluation of several reverse-phase stationary phases has shown that C_{18} columns from various manufacturers provide not only different separation efficiencies, but also different selectivities and retention characteristics.[227,228,229] Such differences between bonded phases are demonstrated in Figure 23 which compares the separation of a standard mixture of FAMEs on the two columns used during this study.

Column 1 was a 30 x 0.4 cm Varian MicroPak CH-10. The functionality of

FIGURE 23 Comparison of the Reverse-Phase HPLC Separation of 18:1(9)c, 18:1(9)t and 18:0 on two C_{18}-Bonded Phase Columns



Column 1:-	MicroPak CH-10 30x0.4 cm
Column 2:-	Waters Associates/Millipore Fatty Acid Analysis Column 30x0.4 cm
Flow Rate:-	1.0 ml/min
Temperature:-	20°C
Gradient:-	60 mins 0-100% CH_3CN

Peak	**Assignment**
1	18:1(9)c
2	18:1(9)t
3	18:0

this column is octadecylsilane and the base material is LiChrosorb Si60. Column 2 was a 30 x 0.4 cm Fatty Analysis Column supplied by Waters Associates/Millipore. No specifications as to the packing of this column are supplied but it is probably μBondapak-C_{18}. If so, the functionality of this column is once again octadecylsilane but in this case, the base material is μPorasil.

In each case, the chromatograms show the separation of a mixture of the methyl esters of 18:1(9)t 18:1(9)c and 18:0. In both cases, 2μl of sample (ca. 10 mg/ml dissolved in methanol) were injected onto the columns after an initial hold time of 0.2 minutes. Both were run as a water/acetonitrile gradient over 60 minutes at 20°C at a flow rate of 1.0 ml/min. 18:1(9)c Eluted first, followed by 18:1(9)t and finally 18:0. Although the efficiencies of the two columns are similar, Column 1 exhibits an enhanced selectivity which was difficult to match with Column 2 even after Simplex optimisation.[210,230] On the basis of this, all subsequent separations were obtained with the Micropak column and a water/acetonitrile solvent system.

The instrument was a Varian 5000 liquid chromatograph interfaced with a Vista 402 data station. The detector was a Varian UV-50 variable wavelength detector, set at 210 nm.

The separation of fatty acids by HPLC has been reported.[222] In such separations, the pH of the mobile phase is adjusted to 2.5 with perchloric acid to suppress ionisation of the acids and provide better retention. In this study the separation of the methyl esters are reported and the pH of the mobile phase is unadjusted.

b) Characterisation of Fatty Acid Methyl Esters by HPLC

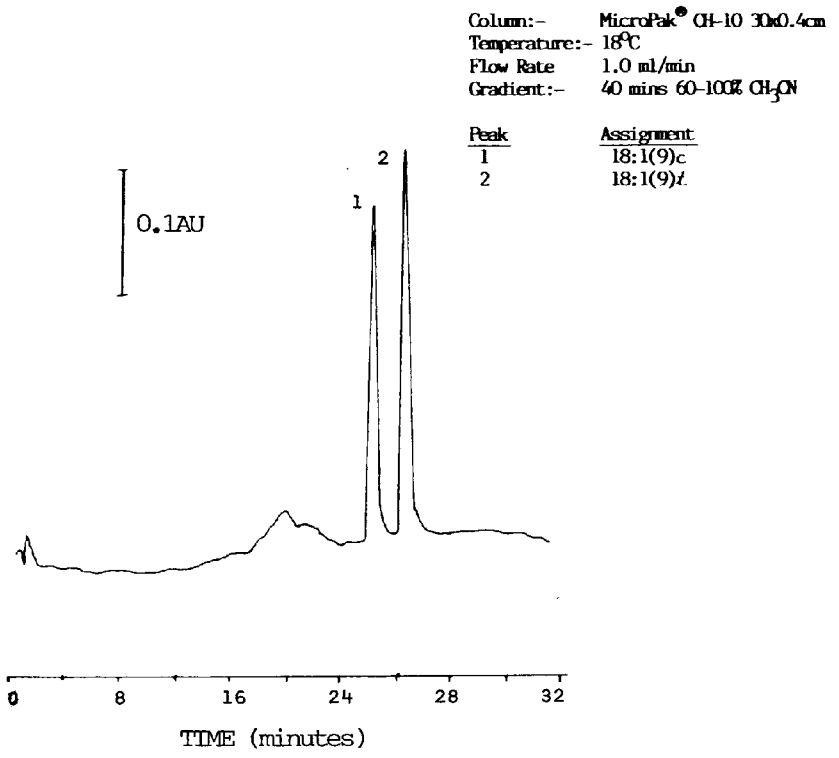
It has already been demonstrated that the separation of FAMEs is possible by reverse-phase HPLC not only by degree of unsaturation, but also by the geometry of the double bond (Figure 23). Such separations are readily achieved by running a simple water/acetonitrile gradient. With optimisation, baseline separation of 18:1(9) *cis* and *trans* is achieved and this compares favourably with that obtained with a packed OV-275 coated column in GLC (Figure 24). The HPLC separation illustrated in Figure 24 was obtained by gradient elution over 40 minutes at 18°C and with an initial solvent composition of 60% acetonitrile.

Initial runs were performed at ambient temperature and separations varied in reproducibility. On running the chromatograms at a range of temperatures between 15-60°C, it was found (surprisingly) that increasing the temperature reduced the resolution. Although increasing the temperature increases column efficiency (by increasing the rate of mass transfer), this is offset by a loss of selectivity. Optimum separation was achieved when the runs were performed at 18°C.

Figure 25 illustrates the reverse-phase HPLC separation of commercially available monoenoic fatty acids as the methyl esters at 20°C. The initial gradient was 0-40% acetonitrile over 30 minutes and thereafter, 40-100% acetonitrile over 60 minutes. This mode of chromatography readily separates FAMEs on the basis of chain length, degree of unsaturation and configuration of the double bond. In addition, the partial separation of positional isomers is also achieved. Esters 18:1(6)c,t; (9)c,t and (11)c,t resolve into four peaks. Esters 18:1(6)c and (11)t co-eluted and 18:1(6)t eluted as an unresolved shoulder on 18:1(9)t.

FIGURE 24 Separation of 18:1(9)c and 18:1(9)t by Reverse-Phase HPLC and Packed Column GLC

a) HPLC



b) GLC

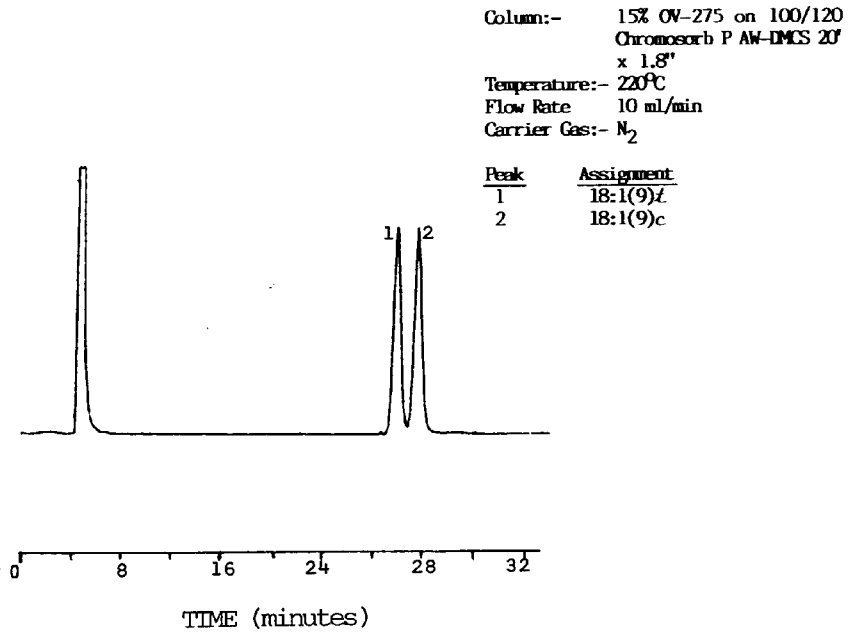
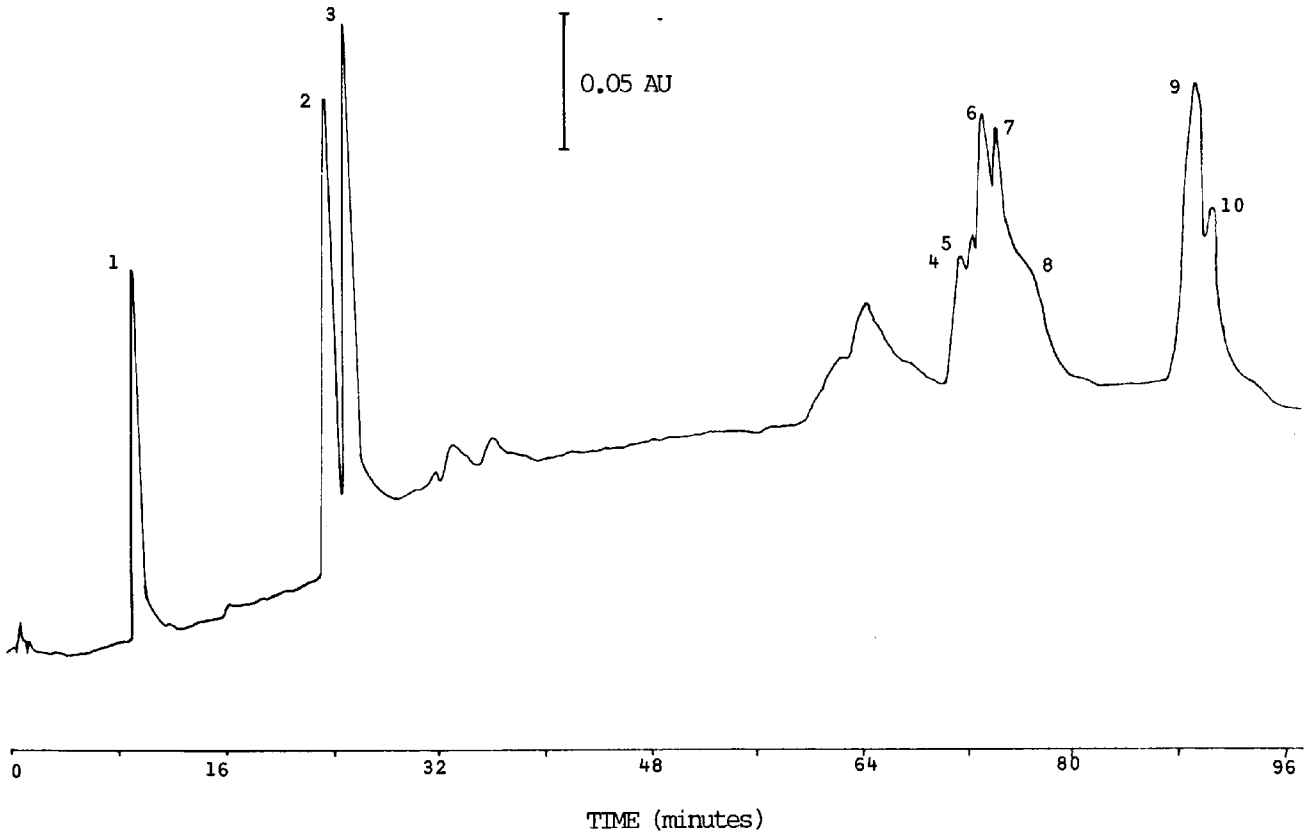


FIGURE 25 Reverse-Phase HPLC Separation of Geometrical and Positional Isomers of Fatty Acid Methyl Esters

Column:- MicroPak® CH-10 30x0.4cm
Temperature:- 20°C
Flow Rate:- 1.0 ml/min
Gradient 1:- 30 mins 0-40% CH_3CN
Gradient 2:- 60 mins 40-100% CH_3CN



Peak	Assignment
1	14:1(9)c
2	16:1(9)c
3	16:1(9)t
4	18:1(11)c
5	18:1(9)c
6	18:1(6)c
	18:1(11)t
7	18:1(9)t
8	18:1(6)t
9	18:0
10	20:1(11)c

Unfortunately, because of instrumentation failure, it was not possible to follow up this initial work. It was thought initially that the problems encountered were attributable to working at 210 nm but derivatisation to the 4-bromophenacyl esters and observation at 254 nm indicated that the problem lay with the instrumentation rather than the method. The separation shown in Figure 25 was not optimised and in view of the observations of Battaglia and Frolich who report the quantitative separation of the (5)-(11)t and (6)-(10)c methyl octadecenoates by HPLC, further improvement may be possible. In this context, the addition of $AgNO_3$ to the mobile phase may prove beneficial. Such techniques have been applied to the separation of 18:1(9) *cis* and *trans* as the phenacyl esters.[231]

An additional point to be considered, in the application of the technique to quantitative analysis is that unsaturated acids have much larger extinction coefficients than the corresponding saturated acids at 210 nm. Consequently therefore, it is imperative to obtain response factors as the sample concentration in the flow cell is related to the fraction of light transmitted through the cell by Beer's law.

To summarise, the following general characteristics are apparent from the reverse-phase HPLC separations of FAMEs. Separation by chain length alone is dramatic, retention increasing with increasing chain length. Increasing unsaturation decreases the retention times of the acids thus, 18:0 elutes after 18:1 etc. The retention of *trans* unsaturated acids are intermediate between the saturated and the *cis* unsaturated acids. Partial separation of positional isomers was achieved, retention decreasing with increasing distance of unsaturation from the carboxyl group.

10 Spectroscopic Characterisation

10.1 Infrared Spectroscopy

a) General IR Features of Fatty Acids

In addition to aliphatic C-H stretching and bending, the most characteristic feature in the IR spectra of fatty acids is the extremely broad OH stretching absorption from 3500 to 2500 cm^{-1}. This band is attributed to the strong hydrogen bonding present in the dimer. The absorption partially obscures C-H stretching that occurs in the same region although the absorption at about 2940 cm^{-1} increases in intensity relative to the OH stretch with increasing chain length.

The strong, sharp carbonyl stretching absorption occurs at a position characteristic of acid dimers at 1712 cm^{-1} when the spectrum is run as a melt but shifts to 1687 cm^{-1} when run in the solid phase. Three other absorptions may be assigned to the carboxylic acid function. Firstly, there is a low to medium intensity band at 1430 cm^{-1} which may be assigned to OH in-plane bending. Secondly, there is the broad C-O stretch between 1300 and 1190 cm^{-1} which generally is as intense as the OH stretching band. When run in the solid phase, this band is broken into a number of sharp peaks which increase in number with increasing chain length. Finally, there is a broad, low to medium intensity OH out of plane bending at 930 cm^{-1} which increases in intensity and shifts to 915 cm^{-1} when run in the solid phase.

The broad intense absorptions because of the free carboxyl group, render free acids unsuitable for purposes of structure determination as vibrations attributable to other functional groups may be fully or partially obscured. It is common practice therefore to subject fatty acids to IR

spectroscopy as the methyl ester derivatives or, in the unesterified state, only if bound to glycerol. Furthermore, although much valuable information about the physical state of lipids can be derived from their spectra in the solid state, most information on the chemical nature of fatty acid derivatives can be obtained when they are in solution, usually carbon tetrachloride or carbon disulphide.

In methyl ester derivatives, OH stretching and bending are absent and the two most characteristic features in the spectrum are the strong C=O and C-O stretching which in solution absorb at 1728 cm^{-1} and 1150-1250 cm^{-1} respectively.

b) Unsaturated Fatty Acids

Standard IR textbooks indicate that double bonds should exhibit an absorption at 1657 cm^{-1} in *cis*, and at 1673 cm^{-1} in *trans* esters. Similarly, triple bond stretching vibrations should result in an IR absorption band at 2250 cm^{-1}. In practice, however, in the acids synthesised, this is not the case and such absorptions are at best very indistinct and in most cases, absent. This results from the local symmetry around the unsaturated bond being sufficiently high to cause the vibrations to become IR-inactive.

The reasoning for this inactivity is that a bond must present an electrical dipole which is changing at the same frequency as the incoming radiation in order for energy to be transferred. The changing electrical dipole of the bond can then couple with the sinusoidally changing electromagnetic field of the incoming radiation. Effectively therefore, bonds which are symmetrically substituted or are adjoined to large groups either side, such as in these long chain monounsaturated fatty acids,

will not absorb in the infrared.

Accordingly, the observations are in agreement with those of Davies et al.[232] who, from spectra of methyl octadecenoates and octadecynoates have reported that bands of reasonable intensity are observed only when unsaturation is (n-1), or when conjugated with a carboxyl group.

In addition, *cis* double bonds give rise to small bands at about 3030 cm^{-1}, but although useful as a diagnostic aid, it is again not sufficiently distinct to be of use in quantitative estimates. In contrast, *trans* double bonds exhibit a sharp band at around 967 cm^{-1}, as a result of the out of plane bending mode of the olefinic hydrogens. Measurement of this absorption under controlled conditions, has long been the basis for a quantitative method adopted by the AOCS for the determination of the *trans* content in lipid mixtures. In the recommended procedure,[200] a standard is run at the same time as the unknown and the amount of *trans* isomer in the latter is calculated from the ratio of absorptivities at 967 cm^{-1} using a baseline drawn from 944 to 998 cm^{-1}.

In an alternative rapid procedure,[233] the ratio of the absorbances at 1162 cm^{-1} (because of the carbonyl function) and at 970 cm^{-1} in the unknown is measured and the amount of *trans* isomer present is obtained by reference to a standard curve. For accuracy with this procedure however, the standard curve must be prepared with material very similar to that to be analysed.[234]

It has been found,[53] that the AOCS baseline, whereas undoubtedly a good compromise, does not make full allowance for variation in background absorption at 970 cm^{-1} because of factors other than the presence of *trans* bonds. This is particularly so in triacylglycerols where non *trans*

containing triacylglycerols exhibit a broad absorption at 976 cm^{-1}. Several proposals to compensate for this have been suggested.[53,235,236] With reference to the analysis of *trans* compounds synthesised in this study, this absorption was not altered markedly by the position of unsaturation along the alkyl chain.

To summarise, IR spectroscopy may be regarded as a valuable method for detecting the presence of *trans* unsaturation in fatty acids and furthermore, for estimating the amount of *trans* unsaturation in lipid mixtures, especially when they are in an esterified form. Nevertheless, other procedures must be used to locate the exact position of the double bond along the alkyl chain. In contrast, acetylenic and *cis*-alkenoic acids (with the exception of cases where unsaturation is terminal or conjugated with the carboxyl) exhibit no distinguishing absorptions that set them apart from IR spectra of saturated acids.

Major absorptions exhibited in the IR spectra of fatty acids and esters, based on data recorded in this study and by Davies et al.,[232] are summarised in Table 31. The IR spectrum of 11-hexadecynoic acid run as a melt is illustrated in Figure 26 and the spectrum of methyl *cis*-8-hexadecenoate is illustrated in Figure 27. The insert illustrates the out of plane bending of the olefinic hydrogens at 967 cm^{-1} in the corresponding *trans* ester.

Finally, it would be prudent to mention that in contrast to infrared, structural features in unsaturated fatty acid methyl esters can give rise to distinctive bands in Raman spectroscopy. Davies et al.[232] report that characteristic bands are found for *cis* double bonds (1656 cm^{-1}), *trans* double bonds (1670 cm^{-1}) and triple bonds (2232 and 2291 cm^{-1}).

TABLE 31

Major IR Absorption Frequencies of a) Fatty Acids and b) Methyl Ester Derivatives

Absorption Frequency (cm^{-1})	Assignment
a) Fatty Acids	
3350-2500	Strong, broad OH stretch.
2966-2854	Strong, sharp C-H aliphatic stretch.
1712	Strong, sharp C=O stretch in solution. Shifts to 1687 cm^{-1} when run as solid.
1465	Sharp, medium to strong CH_2 scissoring.
1430	Medium, OH in plane bending.
1420	Medium, CH_3 symmetrical bending.
1300-1190	Strong, broad C-O stretch. Band is broken into a number of sharp peaks when run as solid.
930	Broad, low to medium O-H out of plane bending. Shifts to 915 cm^{-1} and increases in intensity when spectrum is run as solid.
b) Methyl Esters	
3005	Small, weak =CH stretch in *cis* esters.
2966-2854	As acids.
2250	C≡C Stretch in acetylenic esters when unsaturation is terminal or conjugated to the carboxyl.
1728	Strong, sharp C=O stretch.
1670	C=C Stretch in *trans* esters when unsaturation is terminal or conjugated to the carboxyl.
1657	C=C Stretch in *cis* esters when unsaturation is terminal or conjugated to the carboxyl.
1465	As acids.
1420	As acids.
1250-1150	Strong, broad C-O stretch.
967	Sharp =CH out of plane bending in *trans* esters.

FIGURE 26 IR Spectrum of 11-Hexadecynoic Acid

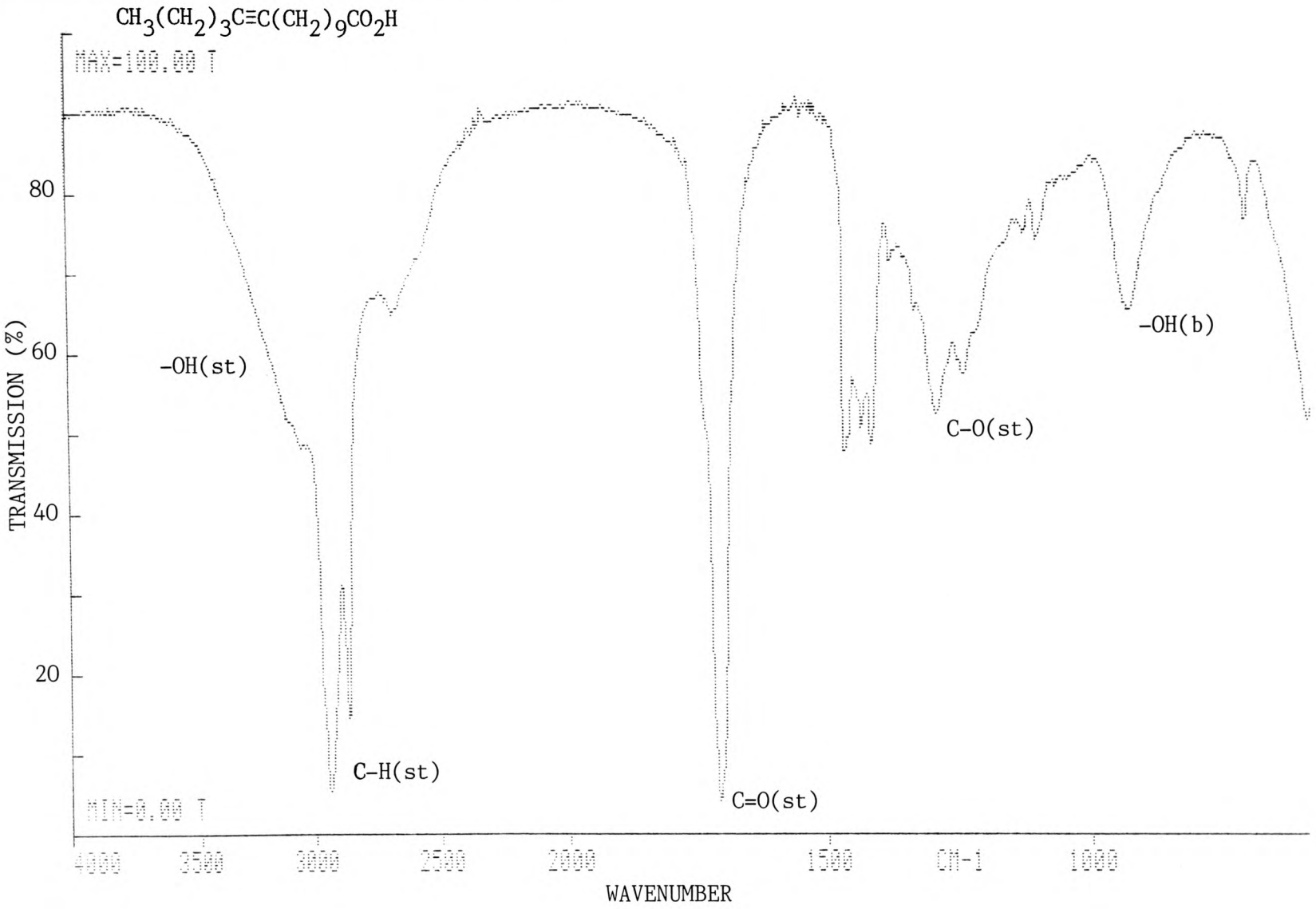
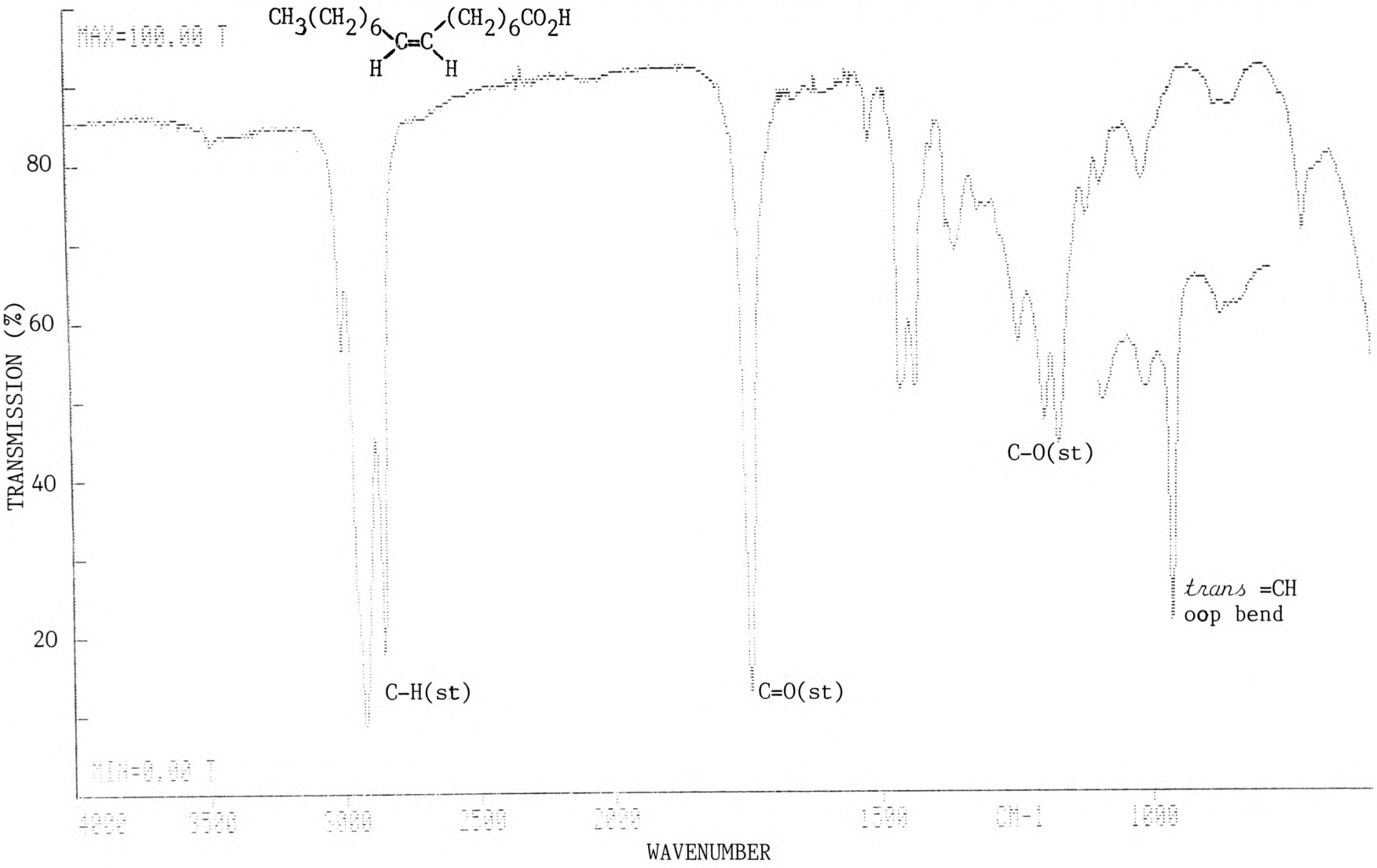


FIGURE 27 IR Spectrum of Methyl *cis*-8-Hexadecenoate
(The insert shows the =CH out of plane bending of the corresponding *trans* isomer at 967 cm^{-1})



Once again however, the position of unsaturation does not affect the spectrum unless it is at either extremity of the molecule.

10.2 NuclearMagneticResonance Spectroscopy

The first accurate determination of unsaturation in lipids by NMR spectroscopy was reported over 24 years ago.[237] Since then, the performance of NMR spectrometers has been greatly improved and NMR spectroscopy, especially ^{1}H but also more recently ^{13}C, has been increasingly applied to the identification of lipid structures.[198,202,203,204]

In this study, ^{1}H and ^{13}C NMR were used to investigate the structural characteristics of the series of synthesised acids. For purposes of comparison, saturated and acetylenic acids and some PUFA are discussed in this section. The ^{17}O spectra of some acids were also recorded.

All spectra were obtained on a Jeol FX-90Q FT-NMR spectrometer operating at 89.55 MHz for ^{1}H, 22.49 MHz for ^{13}C and 12.11 MHz for ^{17}O. For ^{1}H and ^{13}C measurements, fatty acids or their methyl esters were dissolved to ca. 20 mg/ml in $CDCl_3$ and TMS added as internal standard. For ^{17}O, saturated solutions of acids in $CDCl_3$ were used and the spectra recorded relative to D_2O as an external standard.

a) Characterisation of Fatty Acids by 90 MHz ^{1}H NMR Spectroscopy

A wide range of unsaturated fatty acids have been subjected to ^{1}H NMR spectroscopy on 40–100 MHz instruments including the complete series of methyl *cis*-octadecenoates at 60 MHz.[238] Generally however, such spectra obtained with low-resolution CW instruments were of limited value because the fatty acid molecules contain too many protons in very similar chemical environments.

The development of more powerful FT instruments, has enabled the acquisition of more informative spectra and data are available for a wide range of unsaturated fatty acids which include all the *cis* and *trans* octadecenoic acids and methyl esters at 220 MHz.[239]

The ^1H NMR spectra of fatty acids in this study were recorded at 90 MHz. Although providing more information than spectra at 60 MHz, the spectra will not be as informative as those at obtainable at higher field strengths such as 220 MHz. Nevertheless, it is to be expected that the discrimination of isomers is possible at 90 MHz. Spectra of these acids were therefore recorded to determine to what extent the major features were affected by configuration and position of unsaturation and to what extent it would be possible to identify a geometrical and/or positional isomer from its 90 MHz ^1H spectrum.

Before discussing monounsaturated acids, it would be prudent to first examine the corresponding saturated acids as both exhibit common absorbances. Accordingly, ^1H spectra of C_{10}-C_{22} even chain length saturated fatty acids were recorded and the main features are now summarised.

i) Saturated Acids

The 90 MHz ^1H NMR spectrum of a saturated fatty acid exhibits four absorptions. These are the terminal methyl group (0.880 ppm, distorted triplet), the α methylene protons (2.32 ppm, triplet J=7.2 Hz), a large, single absorption (1.255 ppm) for the remaining polymethylene protons and a small absorption for the methylene protons β to the acid group (1.59-1.61 ppm).

The chemical shift of the hydroxyl proton absorbs downfield of 10 ppm although its actual position and intensity may vary as a result of

hydrogen bonding. In contrast, the absorption of the methoxyl group in methyl esters is quite characteristic and absorbs as a sharp singlet at ~3.65 ppm.

^1H chemical shifts for C_{10}-C_{20} saturated fatty acids are summarised in Table 32 and the spectrum of octadecanoic (stearic) acid is illustrated in Figure 28. On increasing chain length, the absorptions of the methyl, α and β protons become increasingly indistinct relative to the polymethylene proton absorption and, in the longer chain length acids, the β proton absorption in particular is barely discernible.

TABLE 32

^1H NMR Chemical Shifts and Assignments of C_{10}-C_{20} Saturated Fatty Acids

$CH_3(CH_2)_nCH_2CH_2COOH$

Acid	Chemical Shift and Assignment (ppm)			
	$-CH_3$	$(CH_2)_n$	β-CH_2	α-CH_2
10:0	0.878	1.265	1.626	2.349
12:0	0.873	1.256	1.604	2.339
14:0	0.880	1.256	1.614	2.347
16:0	0.880	1.256	1.591	2.344
18:0	0.880	1.256	1.618	2.349
20:0	0.880	1.256	1.616	2.352

ii) Monounsaturated Fatty Acids

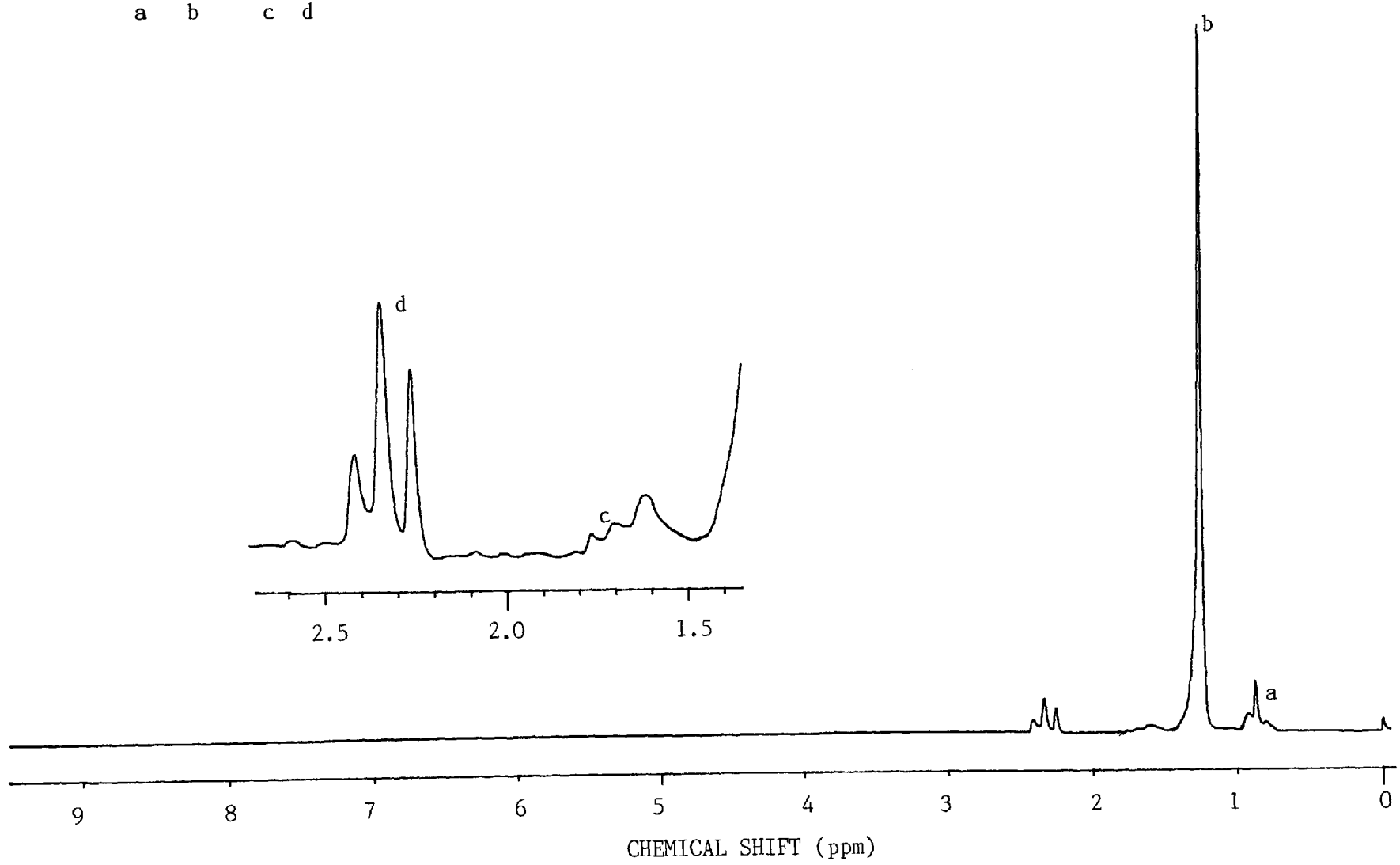
The introduction of an unsaturated bond can result in at least one or two additional absorptions to those found in saturated fatty acids depending on whether unsaturation is acetylenic or olefinic. These arise from the olefinic and allylic protons in alkenoic acids, and propargylic protons in acetylenic acids.

In addition to these readily assigned signals, subtle shifts are induced

FIGURE 28 ^1H NMR Spectrum of Octadecanoic (Stearic) Acid)

$CH_3(CH_2)_{14}CH_2CH_2COOH$

a b c d



in the other absorptions (depending on the type, position and configuration of unsaturation). In particular, the effect of unsaturation, especially an acetylenic bond, on the chemical shift of the alkyl chain protons can be considerable.

Several publications have reported the ^{1}H NMR spectra of acetylenic and alkenoic acids at 60 MHz,[238,241,242,243] and Frost and co-workers have reported the 220 MHz spectra of all the octadecynoic and octadecenoic acids.[239,240,244] It is reported that all 16 positional isomers of octadecynoic acids and all but the 10-, 11- and 12- isomers of octadecenoic acids may be identified from the 220 MHz spectra.

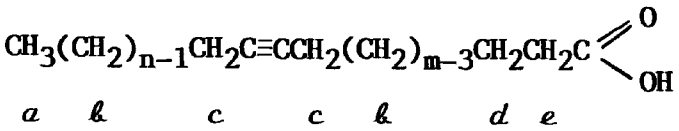
The ^{1}H chemical shifts of acetylenic, *cis* alkenoic and *trans* alkenoic acids are summarised in Tables 33, 34 and 35 respectively and typical spectra are illustrated in Figures 29, 30 and 31. The more salient features of the spectra are now discussed.

^{1}H Spectral Features of Double and Triple Bonds

The presence of olefinic protons is readily signified by the characteristic shift in the region 5-5.5 ppm. This downfield shift compared to protons attached to sp^3 carbons arises partly from the change in hybridisation although more important is the diamagnetic anisotropy generated by the π electrons of the double bond.

Standard textbooks indicate that the configuration of a double bond may be determined from the coupling constant between the olefinic protons. This is always larger for *trans* than for *cis* bonds although actual values are dependent upon the electronegativity of the substituents. For alkyl substituents, typical coupling constants are about 15 Hz (*trans*) and 9 Hz (*cis*).

TABLE 33
^{1}H NMR Chemical Shifts and Assignments of Monounsaturated Acetylenic Acids



Acetylenic Acid[a]	Shift and Assignment(ppm)				
	a	*ℓ*[b]	*c*	*d*	*e*
12:1(5)a	0.898	1.301/1.408	2.140	1.770	2.430
(6)a	0.908	1.313/1.400	2.112	1.681[c]	2.340
(7)a	0.920	1.445	2.101	1.640[c]	2.336
(8)a	0.961[d]	1.430	2.090	1.640	2.334
(9)a	1.093[d]	1.360/1.410	2.102	1.615	2.328
14:1(5)a	0.883	1.283/1.402	2.315	1.751	2.427
(6)a	0.880	1.292/1.461[e]	2.113	1.691[c]	2.345
(7)a	0.890	1.327/1.467[e]	2.100	1.645[c]	2.338
(8)a	0.898	1.410	2.805	1.633	2.333
(9)a	0.920	1.361/1.417[e]	2.081	1.614	2.339
(10)a	0.960[d]	1.342/1.416[e]	2.080	1.614	2.325
(11)a	1.110[d]	1.348	2.100	1.610	2.329
16:1(5)a	0.880	1.273/1.397[e]	2.135	1.781	2.430
(6)a	0.883	1.277/1.437[e]	2.120	1.692[c]	2.345
(7)a	0.880	1.280/1.450[e]	2.101	1.651[c]	2.338
(8)a	0.880	1.291/1.401[e]	2.090	1.631	2.330
(10)a	0.901	1.356	2.079	1.618	2.335
(11)a	0.918	1.295	2.080	1.614	2.328
(12)a	0.960[d]	1.285/1.416	2.080	1.610	2.325
(13)a	1.103[d]	1.320	2.110	1.614	2.327
18:1(7)a	0.880	1.273/1.438[e]	2.091	1.650[c]	2.340
(8)a	0.880	1.280/1.397[e]	2.077	1.631	2.338
(9)a	0.883	1.329	2.080	1.610	2.335
(10)a	0.879	1.321	2.100	1.620	2.326
(12)a	0.887	1.301/1.401[e]	2.090	1.614	2.320
(13)a	0.920	1.275/1.401[e]	2.080	1.614	2.330
(14)a	0.958[d]	1.283/1.411[e]	2.101	1.614	2.324
20:1(9)a	0.879	1.273/1.383[e]	2.091	1.612	2.330
(10)a	0.883	1.330	2.084	1.610	2.326
(11)a	0.880	1.275	2.080	1.614	2.322
(12)a	0.883	1.290	2.090	1.615	2.328
(13)a	0.880	1.331	2.090	1.614	2.340
(14)a	0.901	1.325	2.101	1.621	2.335
(15)a	0.920	1.288/1.400[e]	2.090	1.614	2.336

FOOTNOTES

a) Nomenclature refers to Chain Length:Degree of Unsaturation(Position of Unsaturation)acetylenic Bond.
b) Denotes shift of the main polymethylene proton absorption and anomalous absorptions induced by the deshielding of functional groups.
c) Approximate shift. Absorption partially obscured by anomalous absorptions of the alkyl chain methylene protons.
d) Signal is a well defined triplet.
e) Shoulder on main polymethylene absorption.

TABLE 34

^{1}H NMR Chemical Shifts and Assignments of Monounsaturated *cis*-Alkenoic Acids

$$CH_3(CH_2)_{n-1}CH_2CH{=}CHCH_2(CH_2)_{m-3}CH_2CH_2COOH$$

a *b* *c* *d* *d* *c* *b* *e* *f*

cis-Alkenoic Acid[a]	Shift and Assignment(ppm)					
	a	*b*[b]	*c*	*d*	*e*	*f*
12:1(5)c	0.881	1.278/1.318[c]	2.036	5.356	1.678	2.335
(6)c	0.889	1.300/1.416[c]	2.013	5.342	1.630[d]	2.338
(7)c	0.893	1.323/1.370	2.008	5.322	1.632[d]	2.340
(8)c	0.903[e]	1.348	1.996	5.329	1.621	2.321
(9)c	0.948[e]	1.328	2.001	5.327	1.620	2.320
14:1(5)c	0.881	1.278	2.035	5.355	1.680	2.340
(6)c	0.880	1.275/1.418[c]	2.013	5.341	1.640[d]	2.340
(7)c	0.880	1.300/1.372[c]	2.016	5.331	1.628[d]	2.336
(8)c	0.889	1.288[c]/1.341	2.002	5.329	1.624	2.334
(9)c	0.893	1.322/1.304	1.992	5.327	1.608	2.340
(10)c	0.903[e]	1.335[c]/1.292	1.992	5.329	1.604	2.325
(11)c	0.947[e]	1.300	2.004	5.333	1.600	2.328
16:1(5)c	0.880	1.272	2.031	5.357	1.679	2.340
(6)c	0.880	1.274/1.420	2.013	5.342	1.630[d]	2.335
(7)c	0.880	1.273/1.390[c]	2.004	5.331	1.627[d]	2.339
(8)c	0.880	1.273	1.996	5.327	1.606	2.320
(9)c	0.880	1.304	1.994	5.329	1.612	2.340
(10)c	0.888	1.307	1.996	5.329	1.608	2.324
(11)c	0.894	1.306	1.996	5.329	1.610	2.336
(12)c	0.904[e]	1.304	2.001	5.329	1.610	2.330
(13)c	0.958[e]	1.301	2.005	5.333	1.620	2.339
18:1(6)c	0.880	1.265/1.418[c]	2.014	5.343	1.642[d]	2.340
(7)c	0.880	1.271/1.370[c]	2.005	5.332	1.630[d]	2.329
(8)c	0.880	1.274/1.316[c]	2.001	5.329	1.616	2.341
(9)c	0.880	1.273/1.310[c]	1.996	5.329	1.618	2.340
(10)c	0.880	1.295	1.996	5.329	1.610	2.320
(11)c	0.880	1.288	2.000	5.333	1.610	2.340
(12)c	0.890	1.275	2.000	5.333	1.600	2.332
(13)c	0.894	1.295	1.998	5.326	1.618	2.340
(14)c	0.904[e]	1.287	2.001	5.329	1.620	2.336
20:1(9)c	0.880	1.275/1.311[c]	1.999	5.329	1.611	2.340
(10)c	0.880	1.294	2.001	5.329	1.608	2.336
(11)c	0.880	1.268	1.996	5.329	1.612	2.332
(12)c	0.880	1.285	1.996	5.331	1.612	2.340
(13)c	0.880	1.288	1.996	5.329	1.618	2.340
(14)c	0.881	1.287	2.004	5.329	1.614	2.338
(15)c	0.895	1.286	2.010	5.331	1.615	2.336

FOOTNOTES

a) Nomenclature refers to Chain Length:Degree of Unsaturation(Position of Unsaturation)*cis* Bond.
b) Denotes shift of the main polymethylene proton absorption and anomalous absorptions induced by the deshielding of functional groups.
c) Shoulder on main polymethylene absorption.
d) Approximate shift. Absorption partially obscured by anomalous absorptions of the alkyl chain methylene protons.
e) Signal is a well defined triplet.

TABLE 35

^{1}H NMR Chemical Shifts and Assignments of Monounsaturated *trans*-Alkenoic Acids

$$CH_3(CH_2)_{n-1}CH_2CH{=}CHCH_2(CH_2)_{m-3}CH_2CH_2COOH$$

a *b* *c* *d* *d* *c* *b* *e* *f*

trans-Alkenoic Acid[a]	Shift and Assignment(ppm)					
	a	*b*[b]	*c*	*d*	*e*	*f*
12:1(5)t	0.880	1.284	1.993	5.394	1.662	2.322
(6)t	0.879	1.293/1.390[c]	1.978	5.381	1.618[d]	2.329
(7)t	0.880	1.300[c]/1.334	1.960	5.373	1.611[d]	2.324
(8)t	0.881[e]	1.334	1.948	5.365	1.614	2.340
(9)t	0.930[e]	1.309	1.956	5.366	1.612	2.340
14:1(5)t	0.880	1.273	1.994	5.395	1.665	2.332
(6)t	0.880	1.268/1.438[c]	1.965	5.379	1.612[d]	2.336
(7)t	0.880	1.296/1.372[c]	1.961	5.373	1.612[d]	2.332
(8)t	0.880	1.308	1.948	5.365	1.609	2.320
(9)t	0.880	1.305	1.940	5.365	1.614	2.325
(10)t	0.880[e]	1.371[c]/1.300	1.948	5.369	1.618	2.332
(11)t	0.920[e]	1.300	1.955	5.369	1.610	2.333
16:1(5)t	0.880	1.266	1.994	5.396	1.662	2.336
(6)t	0.879	1.271/1.410[c]	1.965	5.380	1.611[d]	2.330
(7)t	0.880	1.268/1.360[c]	1.956	5.369	1.612[d]	2.315
(8)t	0.880	1.280	1.948	5.365	1.609	2.323
(9)t	0.880	1.300	1.947	5.373	1.614	2.336
(10)t	0.880	1.297	1.940	5.365	1.615	2.325
(11)t	0.880	1.283	1.940	5.369	1.614	2.332
(12)t	0.880[e]	1.384[c]/1.273	1.944	5.369	1.600	2.340
(13)t	0.901[e]	1.280	1.955	5.369	1.618	2.340
18:1(6)t	0.880	1.260/1.400[c]	1.958	5.379	1.619[d]	2.336
(7)t	0.880	1.265/1.360[c]	1.956	5.373	1.630[d]	2.332
(8)t	0.880	1.260/1.290[c]	1.940	5.365	1.612	2.336
(9)t	0.880	1.265	1.940	5.365	1.614	2.330
(10)t	0.879	1.270	1.940	5.365	1.601	2.328
(11)t	0.880	1.278	1.948	5.365	1.615	2.340
(12)t	0.880	1.268	1.940	5.369	1.601	2.320
(13)t	0.880	1.260	1.938	5.369	1.606	2.336
(14)t	0.886[e]	1.276	1.940	5.370	1.616	2.326
20:1(9)t	0.880	1.272	1.940	5.365	1.618	2.341
(10)t	0.880	1.280	1.944	5.365	1.614	2.342
(11)t	0.880	1.263	1.940	5.369	1.600	2.336
(12)t	0.879	1.273	1.940	5.365	1.616	2.336
(13)t	0.880	1.278	1.943	5.365	1.614	2.335
(14)t	0.880	1.267	1.940	5.369	1.614	2.335
(15)t	0.880	1.270	1.940	5.369	1.616	2.339

FOOTNOTES

a) Nomenclature refers to Chain Length:Degree of Unsaturation(Position of Unsaturation)*trans* Double Bond.
b) Denotes shift of the main polymethylene proton absorption and anomalous absorptions induced by the deshielding of functional groups.
c) Shoulder on main polymethylene absorption.
d) Approximate shift. Absorption partially obscured by anomalous absorptions of the alkyl chain methylene protons.
e) Signal is a well defined triplet.

FIGURE 29 ^{1}H NMR Spectrum of 7-Octadecynoic Acid

$CH_3(CH_2)_8CH_2C{\equiv}CCH_2(CH_2)_2CH_2CH_2COOH$

a b c c b d e

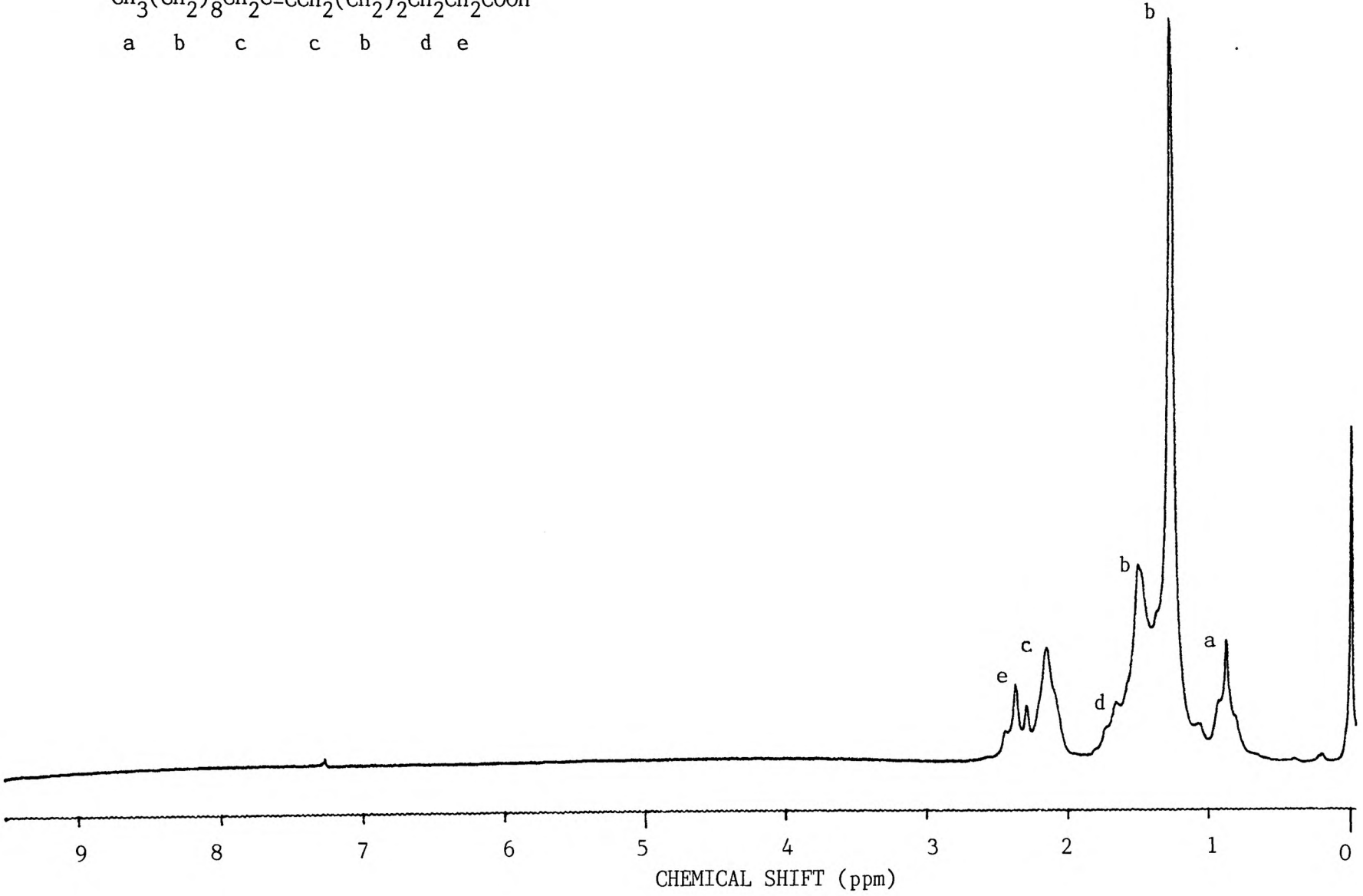


FIGURE 30 ^{1}H NMR Spectrum of *cis*-12-Eicosenoic Acid

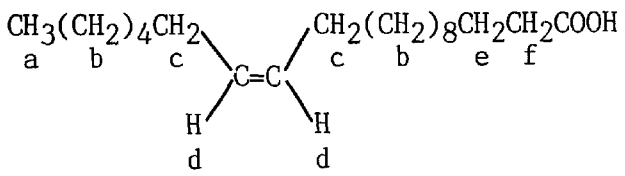
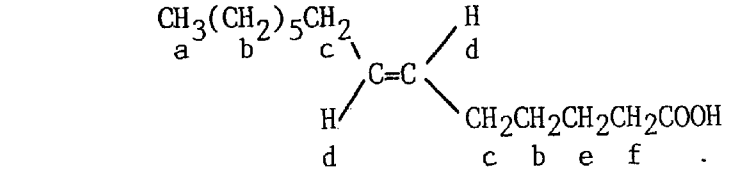
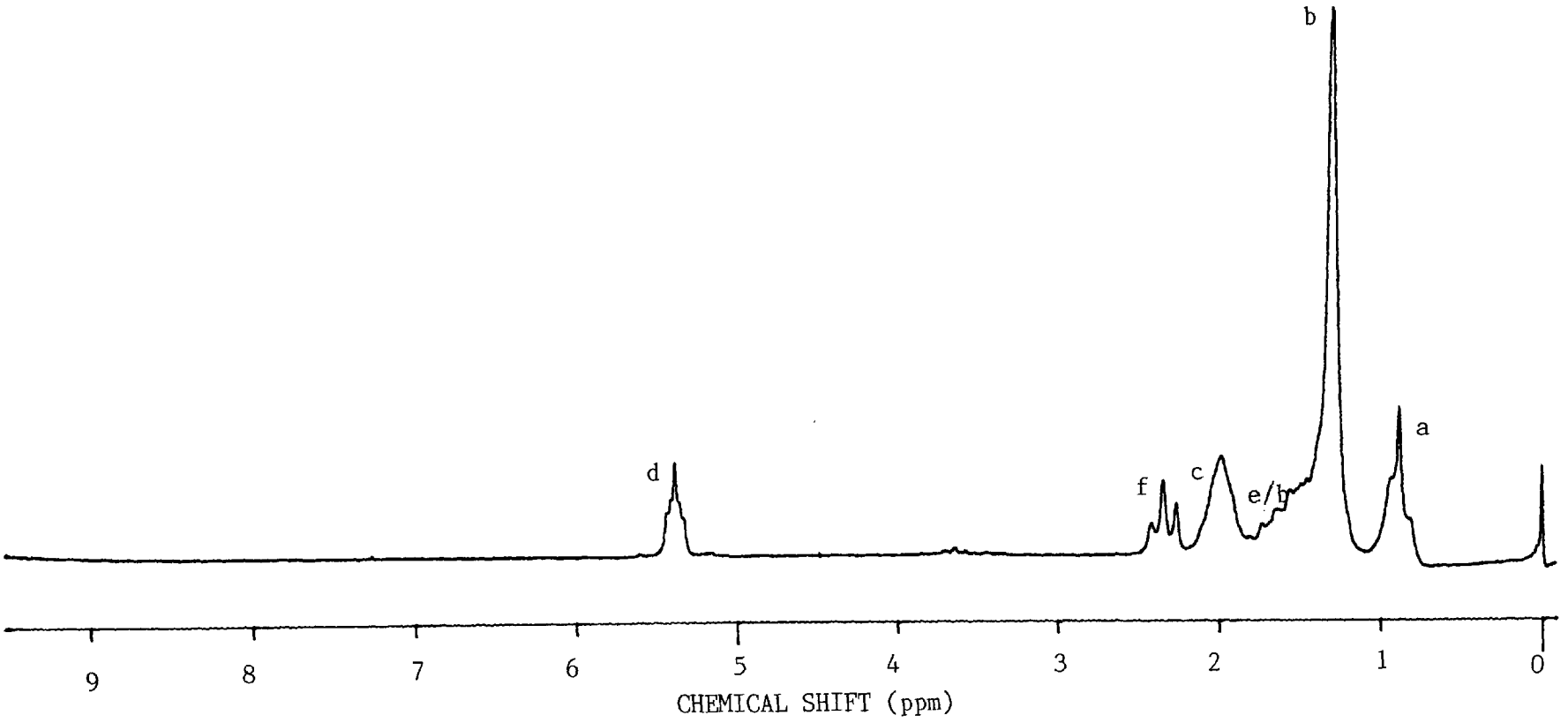


FIGURE 31 ^{1}H NMR Spectrum of *trans*-6-Tetradecenoic Acid





In cases where this coupling constant can be measured, the configuration of the double bond may be unambiguously determined. For most isolated bonds however, this is not possible as the very small differences in chemical shifts between the two olefinic protons produces a complex absorption which is not susceptible to first order analysis. Nevertheless, the different coupling constants involved between olefinic and allylic protons cause isolated *cis* and *trans* double bonds to give different and distinctive absorptions. Consequently, the olefinic protons in *cis* isomers absorb as an apparent triplet (J=3.5 Hz), whereas in *trans* isomers, the splitting pattern is more complex. In addition, *cis* and *trans* isomers exhibit different chemical shifts for the olefinic protons, the *cis* isomer absorbing on average about 0.03 ppm upfield of the corresponding *trans* isomer. For isolated double bonds, the average chemical shifts for *cis* and *trans* alkenoic acids are 5.329 ppm and 5.365 ppm respectively.

The chemical shift of the olefinic proton absorptions are also affected to some extent by the position of unsaturation. As position of unsaturation approaches a functional group, the two olefinic protons can experience slightly different chemical shifts as a result of long range deshielding. The effect of this on the spectrum is a slight downfield shift, and a slightly more complex splitting pattern. This is demonstrated in the 5- and 6- isomers. The chemical shift observed in these cases is the average shift for the olefinic protons. Although they do exhibit different chemical shifts, the difference is not sufficiently large that the absorptions may be regarded as separate at 90 MHz.

Accompanying the olefinic proton absorption is a signal at 1.94-2.01 ppm which may be attributed to the allylic protons. These absorptions are

also dependent upon the configuration of the bond. For isolated *cis* bonds, the chemical shift is about 2.01 ppm. Although itself rather ill-defined, it is noticeably sharper than the corresponding *trans* signal which absorbs about 0.6 ppm upfield at 1.95 ppm.

In acetylenic acids, isolated propargylic protons absorb as a distorted multiplet at 2.08 ppm. This anomalously low shift has been explained in Part Two, Section One, 3 and 4, in terms of diamagnetic anisotropy which is generated by the triple bond. The chemical shifts of allylic protons in *cis* and *trans* alkenoic acids may be similarly explained in terms of diamagnetic anisotropy, the allylic protons lying within the deshielded region in the plane of the bond.

The chemical shift difference between *cis* and *trans* allylic protons may be explained in terms of steric hindrance. In *cis* isomers, these protons spend a large proportion of their time within the plane of the bond (a region of deshielding) and consequently absorb at lower field than in *trans* isomers where the extent of steric hindrance is much less.

It is reported for the spectra of methyl *cis*-octadecenoates at 60 MHz,[238] that with the exception of 18:1(3) and 18:1(4), the signals for allylic protons and protons on the α carbon absorb as a multiplet centred at 2.10 ppm, equivalent to six protons. A similar trend was observed in the spectra of a limited number of acetylenic acids. In contrast, at 220 MHz,[308] separate signals are observed for all allylic and propargylic protons in C_{18} acids up to, and including 18:1(7) and, in no case do the signals for allylic/propargylic and α protons co-absorb.

At 90 MHz absorptions for the α methylene and allylic/propargylic

protons are sufficiently distinct to be regarded as separate. Integration of these signals results in integers equivalent to two and four protons respectively. The α methylene protons absorb as a well defined triplet (J=7.2 Hz) between 2.32 and 2.34 ppm in an isolated system (cf. saturated acids) although a downfield shift is observed in the 5- and 6- isomers (particularly in the acetylenic acids).

The chemical shift of allylic and propargylic protons in the 5-, 6-, 7- and (n-3) isomers are slightly downfield of the shift in other isomers. This is because of the different chemical shifts induced in allylic and propargylic protons either side of the unsaturated bond by the long range deshielding of the carboxyl and methyl groups. At 90 MHz however, these absorptions are not sufficiently distinct to be regarded as separate and what is observed is an average chemical shift.

Effect of Unsaturation on the Chemical Shift of the Terminal Methyl Protons

The protons of the methyl group, when isolated, absorb as a distorted triplet at 0.882 ppm. As an unsaturated bond approaches the methyl end of the molecule, this absorption shifts downfield. The extent to which the signal is shifted depends very much on the type, configuration and position of unsaturation relative to the methyl group. Chemical shifts for methyl protons of different positional isomers for acetylenic, *cis* and *trans* fatty acids are summarised in Table 36.

Whereas long range deshielding of the *cis* and acetylenic bonds extend up to six carbons, that of the *trans* double bond is markedly less, extending only over a distance of three carbons. Furthermore, whereas in the majority of acids, the absorption is a distorted triplet, in (n-3) and (n-4) acids, it is well defined.

TABLE 36

The Effect of Position and Type of Unsaturation on the ^1H Chemical Shift of the Terminal Methyl Proton Absorption

Isomer	No of Examples	Average Chemical Shift (ppm)		
		Acetylenic	*cis*	*trans*
(n-3)	3	1.109	0.949	0.917
(n-4)	4	0.958	0.904	0.880
(n-5)	5	0.921	0.893	0.880
(n-6)	5	0.900	0.886	0.880
(n-≧7)	5	0.881	0.883	0.880

The long range deshielding of unsaturated bonds may in part be explained in terms of diamagnetic anisotropy. In acetylenic bonds, the larger effects are thought to occur as a result of the alkyl substituents "folding back" into the zone of strong deshielding around the axis of the triple bond. The magnitude of deshielding in double bonds depends on the configuration. In the *cis* isomer, the alkyl substituents lie within a region of stronger deshielding and consequently absorb at lower field than in the *trans* isomer where the degree of shielding and steric hindrance is much less.

Shifts observed for C_{18} acids are in agreement with observations at 60[238] and 220 MHz[239,240] with the exception of the chemical shift of (n-3) *trans* acids where a shift of 0.945 ppm is quoted in the literature.

Absorptions Arising From the Methylene Protons of the Alkyl Chain

The chemical shifts of the remaining alkyl chain protons lie between 1.25 and 1.60 ppm for unsaturated acids. For an isolated methylene group in a long alkyl chain, the basic chemical shift is 1.256 ppm (cf.

saturated fatty acids, Table 32). In monounsaturated acids however, chemical shifts of specific methylene protons are dependent on the long range deshielding of the functional groups. These can operate in an additive manner, resulting in changes in the basic chemical shift value. The magnitude of deshielding can be considerable in protons immediately adjacent to functional groups, but smaller effects can extend further along the alkyl chain. From observations at 220 MHz,[239,240] the carboxyl group can affect the chemical shifts of protons up to a distance of 7 carbons, acetylenic and *cis* double bonds up to a distance of 6 carbons, and *trans* double bonds up to a distance of three carbons. The long range deshielding of the methyl group is considerably less, exerting a measurable effect on only the α methylene protons. As substituent effects are additive, where a methylene group is subject to the influence of more than one functional group, the induced shift can be considerable.

Consequently, alkyl chain protons can exhibit several small absorptions between 1.25 ppm and 1.60 ppm in addition to the main polymethylene proton absorption. Earlier work termed these absorptions "anomalous".[242] Williams and Sgoutas subsequently assigned them in acetylenic acids to what they termed "proximal internal" protons[243] i.e. $(CH_2)_n$ in $-C{\equiv}CCH_2(CH_2)_nCH_2COOH$. From observations at 220 MHz,[239] such assignments were considered to be only partially correct and in certain isomers, are contributed to by protons β to unsaturated bonds.

Necessarily, these chemical shifts are influenced by the long range deshielding of the carboxyl and methyl groups and the unsaturated bond. The extent of these absorptions depends on the position of unsaturation. In some acids, where the induced shifts are large, two signals are

clearly apparent and on occasions, the β methylene absorption at 1.61 ppm may be obscured. In some of the shorter chain acids, several absorptions of near equal intensities are apparent (e.g. 14:1(7)a).

Frost and Gunstone report that the shifts induced in the polymethylene protons by functional groups at 220 MHz,[239] result in distinctive absorption patterns for the 18:1 acids which facilitates the identification of positional isomerism. At 90 MHz, insufficient resolution is provided for unambiguous identification in this manner. Whereas several acids may be identified in this manner, most of the longer chain acids only exhibit an anomalous absorption as an unresolved shoulder on the main polymethylene proton signal in the expanded spectrum.

Although accurate assignment of these absorptions to specific protons at 90 MHz is not possible, in comparing acetylenic, *cis* and *trans* acids, several trends are evident. The extent of these absorptions in *cis* acids is intermediate between that in acetylenic, and *trans* alkenoic acids. They are most apparent in acetylenic acids and these trends follow from the long range deshielding exerted by the respective unsaturated bonds.

Between corresponding positional isomers, the average chemical shift of the polymethylene absorption is greatest for acetylenic acids, followed by *cis* acids. The *trans* acids, where the extent of long range deshielding is least, have the chemical shift nearest to that of the saturated acids. This average chemical shift can however, vary for a given chain length depending on the position of unsaturation.

iii) Polyunsaturated Fatty Acids

The ^{1}H shifts and assignments of several commercially available PUFAs are summarised in Table 37. Figure 32 illustrates the spectrum of

TABLE 37

^{1}H NMR Chemical Shifts and Assignments of Some Polyunsaturated Acids

$$\underset{a}{CH_3}\underset{b}{(CH_2)_n}\underset{c}{CH_2}\underset{d}{((CH_2)_y}\underset{e}{CH{=}CH)_x}\underset{c}{CH_2}\underset{b}{(CH_2)_m}\underset{f}{CH_2}\underset{g}{CH_2COOH}$$

Acid[a,b]	Trivial Name	Shorthand Nomenclature	x	y	Shift and Assignment (ppm)						
					a	*b*	*c*	*d*	*e*	*f*	*g*
9,12-Octadecadienoic	Linoleic	18:2(n-6)	2	1	0.890	1.319/1.283[c]	2.043	2.766	5.351	1.616	2.344
9,12-Octadecadienoic[d]	Linelaidic	–	2	1	0.880	1.305/1.265[c]	1.974	2.661	5.397	1.614	2.339
6,9,12-Octadecatrienoic	γ-Linolenic	18:3(n-6)	3	1	0.891	1.314	2.041	2.800	5.350	1.618	2.347
9,12,15-Octadecatrienoic	α-Linolenic	18:3(n-3)	3	1	0.966[e]	1.314	2.071	2.796	5.356	1.614	2.337
4,8,12,15-Octadecatetraenoic	Moroctic	–	4	2	0.974[f]	1.312/1.555	2.100	2.818	5.375	1.649	2.364
5,8,11,14-Eicosatetraenoic	Arachidonic	20:4(n-6)	4	1	0.890	1.395	2.091	2.815	5.361	1.640	2.351

FOOTNOTES

a) All methylene-interrupted except Moroctic acid.

b) Double bond configuration is *cis* unless stated otherwise.

c) Shoulder on main resonance in expanded spectrum.

d) Double bond configuration is *trans*.

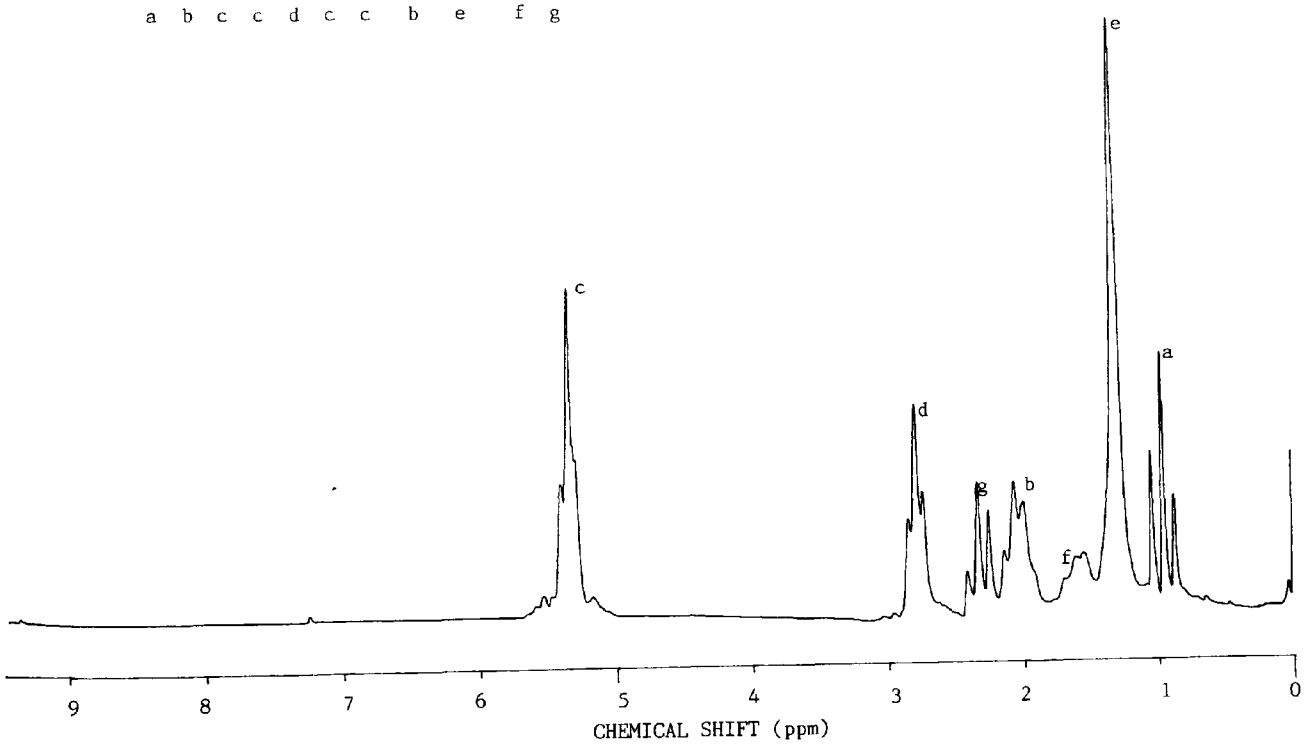
e) J=7.69 Hz

f) J=7.47 Hz

FIGURE 32 ^{1}H NMR Spectrum of α-Linolenic Acid (18:3(n-3))

$CH_3CH_2CH{=}CH(CH_2CH{=}CH)_2CH_2(CH_2)_4CH_2CH_2COOH$

a b c c d c c b e f g



α-linolenic acid (18:1 (n-3)).

Non-conjugated PUFAs, exhibit all the absorptions displayed by mono-unsaturated acids but in addition, there is an absorption attributable to the methylene protons between the double bond(s). Its chemical shift lies between 2.6 and 2.8 ppm, the actual shift depending on the configuration of the double bond and the degree of unsaturation.

In the only *trans* PUFA recorded, linelaidic acid, the chemical shift (2.661 ppm), is about 0.1 ppm upfield of the absorption in the corresponding *cis* acid, linoleic acid (2.766 ppm).

Furthermore, as with allylic and olefinic signals, this absorption exhibits different splitting patterns for *cis* and *trans* acids because of the different coupling constants involved. For *trans* acids, the absorption is an indistinguishable multiplet whereas for *cis* acids, it is apparently a triplet.

It appears from these limited number of spectra, that this signal shifts downfield by about 0.03 ppm with each increase in the degree of unsaturation, absorbing at 2.766, 2.796 and 2.818 ppm for 18:2, 18:3 and 18:4 respectively. Integration of this, and the olefinic proton signal reflects the number of protons contributing to these absorptions and hence, the degree of unsaturation.

Further comparison of linoleic and linelaidic acids indicates that the differences between *cis* and *trans* isomers in monounsaturated acids, with respect to the chemical shifts and splitting patterns of the olefinic, allylic and polymethylene protons, are also apparent in PUFAs.

The influence of position of unsaturation on the chemical shift of the

methyl group is the same as in monounsaturated acids, but it is not affected by the degree of unsaturation. In linoleic (0.89 ppm) and linelaidic (0.88 ppm) acids, the chemical shifts are comparable with corresponding shifts in (n-6) monounsaturated acids (Table 36). γ-Linolenic and arachidonic acids, also (n-6) acids, absorb at 0.89 ppm. α-Linolenic and moroctic acids (n-3), exhibit well defined triplets at 0.966 ppm (J=7.69 Hz) and 0.974 ppm (J=7.47 Hz) respectively.

Although it is possible to identify trends arising from the geometry and degree of unsaturation from these spectra, information which may be determined as a result of position of unsaturation is limited. The ^{1}H NMR spectra of a number of PUFAs at 60 MHz and 220 MHz have been reported however. Christie and Holman have recorded the 60 MHz spectra of all the non-conjugated *cis,cis*-octadecadienoic acids.[119] The spectra of the 6,9- to 10,13- isomers exhibited no distinguishing characteristics that allow their identification. By comparison, the 2,5- to 5,8- and 11,14- to 14,17- isomers all exhibited characteristics which allowed identification at 60 MHz.

Frost and co-workers have recorded the spectra of a number of octadecadienoic acids at 220 MHz and have concluded that most can be characterised by this technique.[239,240] A few however, show only very small differences and the use of shift reagents in these cases is recommended.

b) Characterisation of Fatty Acids by ^{13}C NMR Spectroscopy

There has recently been great interest in the ^{13}C NMR spectroscopy of lipids as the greater sensitivity of ^{13}C NMR spectra to chemical environment make these more informative than ^{1}H spectra despite the low

natural abundance (1.108%) of ^{13}C. Early work report the spectra of several naturally occurring acids and esters although these suffered the disadvantage of a lack of sensitivity, requiring approximately 0.3-0.5g of acid for a usable spectrum.[245,246,247,248] With improvements in instrumentation and data accumulation, the method has been increasingly applied to the analysis of lipids and data are available for many compounds including naturally occurring lipid mixtures,[202,203,204] all the *cis* octadecenoic acids,[249] the methyl esters[249,250,251] and some *trans* octadecenoic acids.[249]

The theory of ^{13}C shielding is well covered in a number of textbooks and is discussed briefly in its broadest terms in Part Two, Section One, 1. ^{13}C chemical shifts are to a large degree additive (especially in unbranched chains). Assuming that the influence of a functional group on the shift of neighbouring carbons is consistent throughout a range of structures, any fatty acid spectrum can be considered as a modification of the spectrum of the long chain alkane. This is because of firstly, the polar head group and secondly, the addition of various substituents (unsaturated bonds) along the chain.

In seeking to interpret the spectrum of an unsaturated acid in terms of its structure, it is necessary to consider a) new signals not present in the saturated acid spectra which are indicative of additional functional groups, b) any change in the chemical shift of easily assigned signals associated with carbon atoms C-1 to C-3 and (n-1) to (n-3) (see below) and c) more subtle differences in the range below 29.9 ppm.

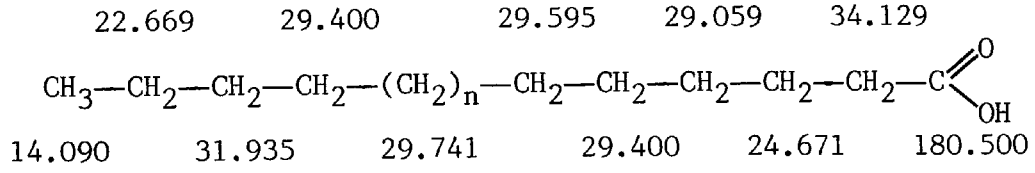
For comparative purposes, ^{13}C NMR spectra of a series of saturated fatty acids were recorded to determine the substituent effects of the carboxyl

and terminal methyl groups in isolated systems. The salient features of these spectra are now summarised.

i) Saturated Fatty Acids

^{13}C chemical shifts of even chain C_{10}-C_{22} saturated fatty acids are summarised in Table 38 and Figure 33 illustrates the spectrum of hexadecanoic (palmitic) acid. There are six clearly separated signals which, on the basis of the work of Grant,[252] Lippmaa[145,253] and Roberts[254] may be assigned to carbons at the carbonyl and methyl ends of the molecule (C-1 to C-3 and (n-1) to (n-3)). In addition, there is a complex group of absorptions between 29 and 30 ppm where it is possible to distinguish several other signals. These signals may be readily allocated to the remaining methylene carbons of the alkyl chain.

Deshielding exerted by end groups extends further than C-3 and (n-3) so that absorptions for C-4, C-5, C-6 and (n-4) may also be readily assigned. Smaller influences such as electric field effects extend beyond these limits so that methylene groups are not insulated from the effects of either (or both) end groups until the C_{14} acid. In addition, these smaller effects may cause the $(CH_2)_n$ chemical shift in shorter chain length acids to be lower than its normal value (see Table 38 and below). Based on chemical shifts presented in Table 38, the following average values ($\pm$0.05 ppm) from the C_{14}-C_{22} saturated acids may be allocated:



In the methyl esters, the chemical shift of the carbonyl carbon is

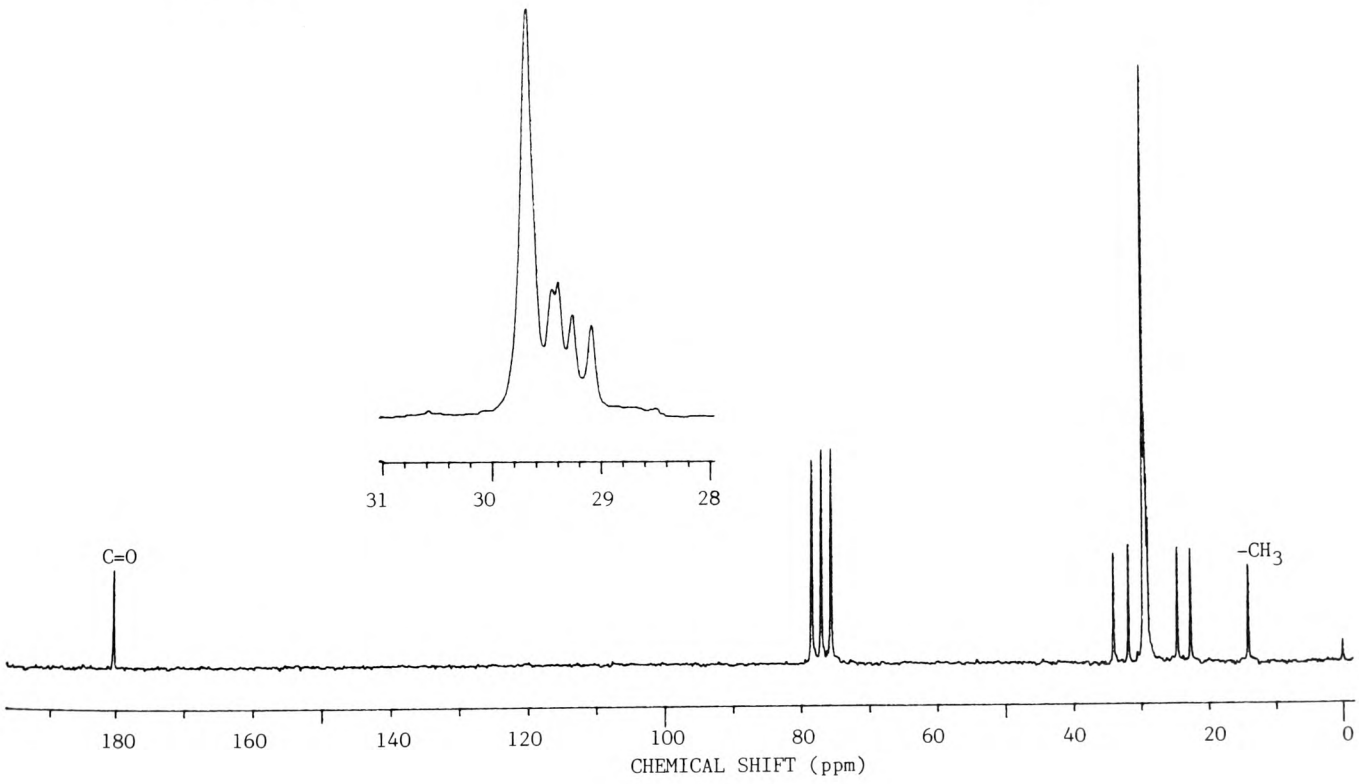
TABLE 38

^{13}C NMR Chemical Shifts and Assignments C_{10}–C_{20} Saturated Fatty Acids

Acid	Shift and Assignment (ppm)																			
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
10:0	180.45	34.08	24.67	29.06	29.25	29.25	29.25	31.84	22.67	14.09	–	–	–	–	–	–	–	–	–	–
12:0	180.50	34.13	24.67	29.06	29.35	29.60	29.60	29.60	29.35	31.89	22.67	14.09	–	–	–	–	–	–	–	–
14:0	180.45	34.13	24.67	29.06	29.40	29.60	29.64	29.64	29.64	29.64	29.40	31.94	22.72	14.09	–	–	–	–	–	–
16:0	180.35	34.13	24.67	29.06	29.40	29.60	29.69	29.69	29.69	29.69	29.69	29.69	29.40	31.94	22.69	14.09	–	–	–	–
18:0	180.21	34.08	24.67	29.06	29.40	29.60	29.74	29.74	29.74	29.71	29.74	29.74	29.74	29.74	29.40	31.94	22.72	14.12	–	–
20:0	180.45	34.13	24.67	29.06	29.40	29.59	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.40	31.94	22.67	14.09

FIGURE 33 ^{13}C NMR Spectrum of Hexadecanoic (Palmitic) Acid

$CH_3(CH_2)_{14}COOH$



upfield of its position in the acids at ~174 ppm and there is an additional signal at ~51.3 ppm because of the methyl carbon of the methoxy group. Furthermore, the chemical shifts of other signals from C-2 onwards are modified slightly as a result of the slightly different long range deshielding exerted by the methyl ester head group.

Generally, for many of the shorter chain length molecules (particularly those which incorporate an unsaturated bond) the number of signals is the same as the number of carbon atoms and the allocation of signals to individual carbon atoms is not difficult. The information so provided about shielding and deshielding influences can then be transferred to the spectrum of longer chain length acids. In such cases, the number of signals is less than the number of carbon atoms and some absorptions in the 28-29 ppm region can apply to more than one carbon atom. The incomplete resolution in such spectra limits the accuracy of some of the assignments and in the most difficult of cases some assignments are in doubt and may even be incorrect.

In ^{13}C NMR, peak heights for one carbon atom is seldom identical and it would be imprudent to assign the correct number of carbon atoms to overlapping absorptions from peak heights alone. Nevertheless, assessing the influence of the various functional groups upon one another and adjacent methylene carbons, facilitates the use of ^{13}C NMR for structural analysis.

ii) Monounsaturated Fatty Acids

The presence of unsaturated centres in the alkyl chain provide further signals associated with the new group and causes shifts in the signals observed in saturated acids of the same length. These shifts are in most

cases sufficient to indicate both the nature and position of the functional group. Typical spectra are illustrated in Figures 34, 35 and 36. ^{13}C chemical shifts of commercially available and synthesised acetylenic and alkenoic acids are summarised in Tables 39, 40 and 41. Major features of the spectra are now discussed.

Unsaturated Carbon Atoms

The assignment of unsaturated carbon absorptions in a ^{13}C spectrum is relatively simple as the shifts fall within three broad ranges depending on the state of hybridisation. The general trend is $sp^3 > sp > sp^2$ and, as discussed in Part Two, Section One, 3, this downfield shift in ^{13}C is principally attributable to an increase in local paramagnetic shielding. For most acetylenic acids, this results in acetylenic carbons exhibiting two absorptions at around 80 ppm. For alkenoic acids, the chemical shifts of unsaturated carbons are downfield of the acetylenic carbon atoms at around 130 ppm. Two signals are apparent in all the C_{12} and C_{14} acids, but in the longer chain length acids, some isomers exhibit only one signal. These are the 16:1(11), 18:1(12)-(13) and 20:1(12)-(15) acids. In these cases, the unsaturated carbons are uninfluenced by long range deshielding of the carboxyl and methyl groups. For acetylenic acids, the chemical shift of these carbons is about 80.19 ppm. In the *cis* alkenoic acids, the shift is about 129.82 ppm. The shift of *trans* acids in general are on average about 0.4 ppm downfield of the *cis* analogue and when isolated, is about 130.32 ppm. It is apparent therefore that unsaturated carbons are influenced by the carboxyl group up to C-11 and by the terminal methyl group up to (n-4) and, in appropriate short chain acids (e.g. 14:1(10)), by both groups in an additive manner.

FIGURE 34 ^{13}C NMR Spectrum of 7-Tetradecynoic Acid

$CH_3(CH_2)_5C{\equiv}C(CH_2)_5COOH$

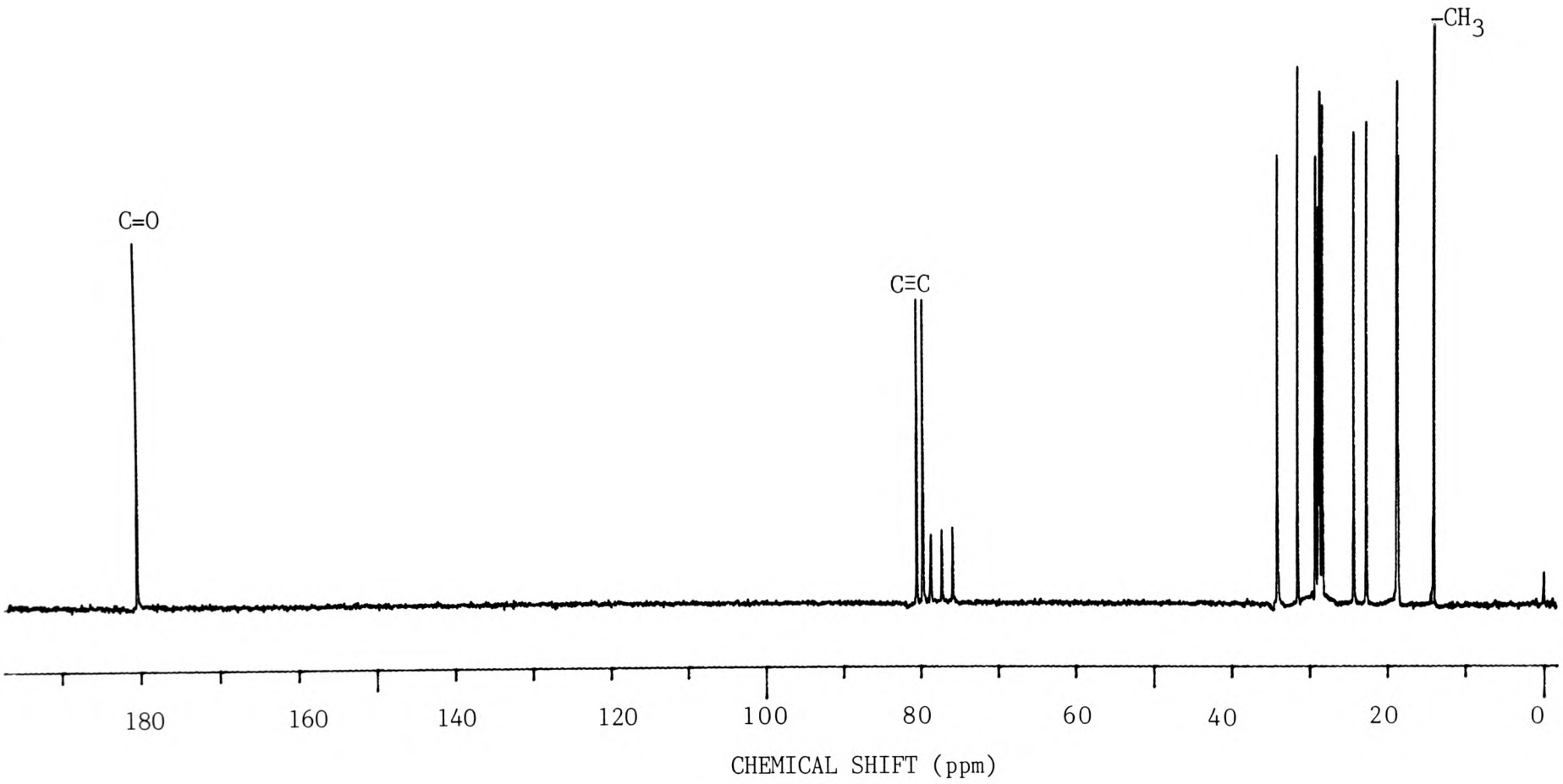


FIGURE 35 ^{13}C NMR Spectrum of *cis*-14-Eicosenoic Acid

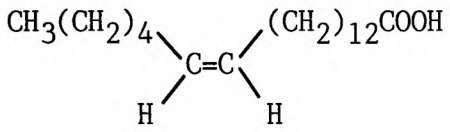


FIGURE 36 ^{13}C NMR Spectrum of *trans*-10-Hexadecenoic Acid

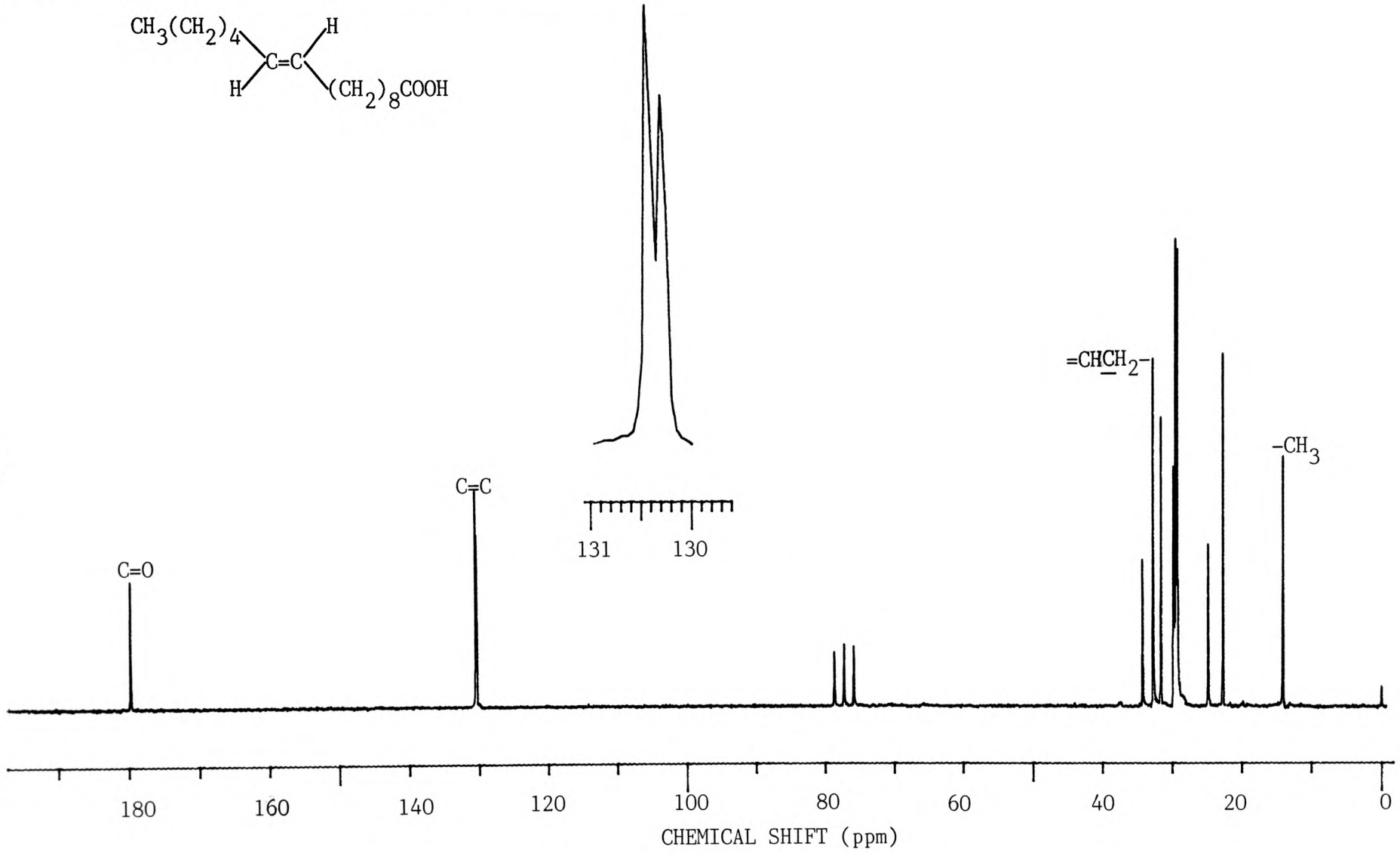


TABLE 39

^{13}C NMR Chemical Shifts and Assignments of Monounsaturated Acetylenic Acids

Acid[a]	Shift and Assignment(ppm)																			
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
12:1(5)a	180.05	33.13	24.09	18.30	78.65	81.55	18.72	29.06	28.62	31.45	22.61	14.09	-	-	-	-	-	-	-	-
(6)a	180.45	33.64	23.93	28.51	18.53	79.33	80.79	18.72	28.86	31.20	22.23	13.99	-	-	-	-	-	-	-	-
(7)a	180.45	34.01	24.23	28.33	28.82	18.67	79.72	80.50	18.48	31.35	22.04	13.65	-	-	-	-	-	-	-	-
(8)a	180.02	34.03	24.55	28.69	28.51	29.01	18.72	80.05	80.21	20.77	22.53	13.51	-	-	-	-	-	-	-	-
(9)a	180.33	34.13	34.69	29.06	28.86	28.69	29.16	18.77	79.38	81.65	12.50	14.41	-	-	-	-	-	-	-	-
14:1(5)a	180.01	33.13	24.03	18.31	78.63	81.53	18.77	29.21	28.51	29.21	29.21	31.89	22.67	14.09	-	-	-	-	-	-
(6)a	180.28	33.64	23.94	28.52	18.53	79.38	80.84	18.82	29.21	28.91	28.91	31.84	22.72	14.09	-	-	-	-	-	-
(7)a	180.45	34.02	24.23	28.33	28.82	18.67	79.72	80.50	18.82	29.21	28.67	31.50	22.61	14.09	-	-	-	-	-	-
(8)a	180.26	33.93	24.57	28.57	28.38	28.86	18.72	79.91	80.45	18.72	28.86	31.20	22.23	13.99	-	-	-	-	-	-
(9)a	180.45	34.10	24.64	28.91	28.91	28.82	29.25	18.74	80.03	80.32	18.45	31.57	22.01	13.63	-	-	-	-	-	-
(10)a	180.52	34.18	24.77	29.06	29.16	29.06	28.77	29.16	18.77	80.30	80.11	20.82	22.57	13.51	-	-	-	-	-	-
(11)a	180.45	34.13	24.67	29.06	29.35	29.35	29.21	28.69	29.21	18.77	79.54	81.61	12.49	14.41	-	-	-	-	-	-
16:1(5)a	180.41	33.14	24.08	18.31	78.63	81.55	18.72	29.30	28.96	29.30	29.64	29.64	29.45	31.94	22.67	14.10	-	-	-	-
(6)a	180.18	33.59	23.96	28.52	18.52	79.38	80.34	18.72	29.30	28.96	29.30	29.30	29.30	31.94	22.67	14.09	-	-	-	-
(7)a	180.25	34.02	24.23	28.32	28.91	18.67	79.71	80.49	18.72	29.37	29.06	29.37	29.37	31.93	22.72	14.10	-	-	-	-
(8)a	180.54	34.08	24.52	28.62	28.62	29.42	18.77	79.96	80.44	18.77	29.69	28.91	28.91	31.83	22.67	14.09	-	-	-	-
(10)a	180.11	34.08	24.67	29.11	29.30	29.11	28.72	29.72	18.72	80.12	80.31	18.72	28.86	31.20	22.23	13.99	-	-	-	-
(11)a	180.21	34.12	24.67	29.06	29.35	29.35	29.16	28.86	29.16	18.72	80.16	80.16	18.48	31.35	21.99	13.65	-	-	-	-
(12)a	180.12	34.08	24.67	29.06	29.40	29.40	29.40	29.16	28.86	29.16	18.72	80.35	80.01	20.82	22.57	13.51	-	-	-	-
(13)a	180.23	34.18	24.67	29.11	29.31	29.54	29.54	29.54	29.31	28.86	29.31	18.72	79.56	81.54	12.50	14.41	-	-	-	-
18:1(7)a	180.01	33.98	24.23	28.28	28.77	18.67	79.72	80.55	18.72	29.21	28.86	29.21	29.60	29.60	29.35	31.94	22.67	14.09	-	-
(8)a	180.16	34.13	24.55	28.62	28.62	29.21	18.72	79.91	80.40	18.72	29.35	28.86	29.35	29.59	29.35	31.93	22.72	14.09	-	-
(9)a	180.10	34.12	24.64	29.11	28.97	28.82	29.35	18.72	80.01	80.30	18.72	29.35	29.11	29.40	29.35	31.94	22.72	14.10	-	-
(10)a	180.03	34.08	24.67	29.06	29.21	29.06	28.86	29.21	18.72	80.11	80.30	18.72	29.21	28.86	28.86	31.79	22.62	14.09	-	-
(12)a	180.48	34.12	24.67	29.21	29.40	29.55	29.55	29.21	28.86	29.21	18.72	80.16	80.16	18.72	28.86	31.16	22.23	13.99	-	-
(13)a	180.18	34.13	24.67	29.16	29.40	29.40	29.21	29.40	29.21	28.86	29.21	18.72	80.16	80.16	18.48	31.30	21.94	13.65	-	-
(14)a	180.50	34.08	24.76	29.06	29.45	29.45	29.64	29.64	29.64	29.26	28.86	29.26	18.77	80.38	80.04	20.82	22.59	13.51	-	-
20:1(9)a	180.08	34.08	24.68	28.94	29.40	28.86	29.40	18.72	80.06	80.35	18.72	29.59	28.86	29.59	29.59	29.59	29.40	31.94	22.67	14.90
(10)a	180.50	34.12	24.72	29.06	29.31	29.17	29.06	29.31	18.72	80.12	80.30	18.72	29.31	29.06	29.31	29.31	29.31	31.93	22.72	14.10
(11)a	180.11	34.18	24.67	29.25	29.25	29.25	29.45	29.25	29.45	18.77	80.11	80.21	18.77	29.45	28.96	29.45	29.25	31.94	22.72	14.09
(12)a	180.16	34.18	24.72	29.21	29.45	28.86	29.21	29.21	28.86	29.21	18.77	80.21	80.21	18.77	29.21	28.86	28.86	31.84	22.67	14.09
(13)a	180.47	34.13	24.64	29.06	29.45	29.45	29.45	29.45	29.45	28.88	29.31	18.72	80.20	80.20	18.72	29.31	28.88	31.45	22.61	14.09
(14)a	180.14	34.13	24.76	29.01	29.31	29.64	29.64	29.64	29.64	29.64	28.86	29.31	18.77	80.16	80.16	18.77	28.86	31.94	22.23	13.99
(15)a	180.20	34.18	24.67	29.01	29.35	29.69	29.69	29.69	29.69	29.69	29.69	28.16	28.86	18.72	80.21	80.21	18.48	31.35	22.00	13.65

FOOTNOTES

a) Nomenclature refers to:- Chain Length:Degree of Unsaturation(Position of Unsaturation)acetylenic Bond

TABLE 40

^{13}C NMR Chemical Shifts and Assignments of *cis*-Alkenoic Acids

Acid[a]	Shift and Assignment(ppm)																				
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
12:1(5)c	180.15	33.49	24.61	26.51	128.12	131.34	27.30	29.64	29.06	31.79	22.63	14.09	–	–	–	–	–	–	–	–
(6)c	180.60	34.00	24.30	29.06	26.86	128.98	130.54	27.30	29.41	31.47	22.49	14.10	–	–	–	–	–	–	–	–
(7)c	180.60	34.18	24.67	28.77	29.40	27.01	129.40	130.18	27.01	31.98	22.33	13.99	–	–	–	–	–	–	–	–
(8)c	180.48	34.12	24.68	29.06	28.97	29.60	27.25	129.82	129.76	29.69	22.87	13.80	–	–	–	–	–	–	–	–
(9)c	180.39	34.13	24.67	29.06	29.34	29.06	29.60	27.21	129.16	131.61	20.55	14.40	–	–	–	–	–	–	–	–
14:1(5)c	180.47	33.50	24.57	26.62	128.11	131.35	27.30	29.74	29.40	29.64	29.40	31.98	22.72	14.09	–	–	–	–	–	–
(6)c	180.50	34.08	24.33	29.11	26.87	128.96	130.52	27.30	29.84	29.35	29.35	31.93	22.72	14.09	–	–	–	–	–	–
(7)c	180.49	34.08	24.62	28.77	29.40	27.01	129.35	130.18	27.30	29.79	29.60	31.78	22.72	14.09	–	–	–	–	–	–
(8)c	180.50	31.13	24.67	29.06	28.96	29.40	27.52	129.56	130.06	27.25	29.40	31.49	22.49	14.09	–	–	–	–	–	–
(9)c	180.49	34.13	24.67	29.06	29.16	29.06	29.69	27.21	129.74	129.99	26.96	31.98	22.28	13.99	–	–	–	–	–	–
(10)c	180.52	34.08	24.67	29.11	29.30	29.30	29.30	29.74	27.21	129.99	129.60	29.74	22.87	13.80	–	–	–	–	–	–
(11)c	180.31	34.13	24.67	29.16	29.30	29.47	29.47	29.30	29.69	27.25	129.27	131.51	20.60	14.41	–	–	–	–	–	–
16:1(5)c	180.50	33.49	24.61	26.51	128.12	131.34	27.52	29.64	29.40	29.64	29.64	29.64	29.40	31.94	22.72	14.09	–	–	–	–
(6)c	180.33	33.99	24.30	29.06	26.86	128.91	130.57	27.30	29.64	29.41	29.64	29.64	29.41	31.94	22.69	14.10	–	–	–	–
(7)c	180.50	34.12	24.67	28.82	29.40	27.06	129.37	130.21	27.30	29.74	29.40	29.60	29.40	31.93	22.72	14.09	–	–	–	–
(8)c	180.50	34.23	24.82	29.11	28.96	29.64	27.28	129.60	130.08	27.30	29.84	29.25	29.25	31.98	22.77	14.09	–	–	–	–
(9)c	180.47	34.08	24.67	29.05	29.47	29.05	29.69	27.20	129.69	129.99	27.20	29.69	29.05	31.79	22.72	14.09	–	–	–	–
(10)c	180.50	34.08	24.67	29.21	29.40	29.40	29.60	29.84	27.30	129.79	129.94	27.30	29.40	31.49	22.51	14.10	–	–	–	–
(11)c	180.08	34.13	24.69	29.08	29.28	29.47	29.47	29.28	29.76	27.25	129.69	129.69	27.01	31.83	22.25	13.99	–	–	–	–
(12)c	180.50	34.12	24.67	29.11	29.40	29.40	29.60	29.60	29.40	29.60	27.30	130.06	129.56	29.69	22.87	13.80	–	–	–	–
(13)c	180.46	34.18	24.72	29.11	29.40	29.64	29.69	29.64	29.64	29.40	29.69	27.30	129.30	131.49	20.60	14.38	–	–	–	–
18:1(6)c	180.25	33.98	24.28	29.06	26.86	128.91	130.57	27.25	29.69	29.35	29.69	29.69	29.69	29.69	29.35	31.93	22.72	14.09	–	–
(7)c	180.42	34.12	24.64	28.82	29.47	27.05	129.37	130.21	27.30	29.61	29.47	29.69	29.69	29.69	29.47	31.94	22.72	14.09	–	–
(8)c	180.40	34.13	24.67	29.06	28.97	29.41	27.25	129.60	130.04	27.25	29.69	29.41	29.69	29.69	29.41	31.93	22.68	14.10	–	–
(9)c	180.50	34.13	24.67	29.06	29.35	29.35	29.69	27.21	129.69	129.99	27.21	29.69	29.35	29.55	29.35	31.94	22.72	14.09	–	–
(10)c	180.50	34.18	24.72	29.16	29.35	29.35	29.35	29.74	27.30	129.79	129.94	27.30	29.74	29.35	29.35	31.94	22.72	14.09	–	–
(11)c	180.47	34.08	24.67	29.06	29.40	29.25	29.25	29.40	29.74	27.21	129.84	129.93	27.21	29.74	29.06	31.79	22.67	14.09	–	–
(12)c	180.50	34.13	24.67	29.23	29.38	29.52	29.52	29.52	29.38	29.52	27.23	129.82	129.82	27.23	29.38	31.50	22.51	14.09	–	–
(13)c	180.25	34.23	24.68	29.11	29.35	29.64	29.35	29.64	29.64	29.35	29.69	27.25	129.82	129.82	26.99	31.88	22.28	13.99	–	–
(14)c	180.34	34.08	24.67	29.11	29.40	29.69	29.69	29.69	29.69	29.69	29.40	29.74	27.30	130.06	129.56	29.74	22.86	13.80	–	–
20:1(9)c	180.58	34.22	24.67	29.06	29.30	29.25	29.69	27.30	129.60	129.99	27.30	29.74	29.40	29.59	29.69	29.69	29.40	31.93	22.68	14.09
(10)c	180.54	34.13	24.68	29.21	29.41	29.60	29.41	29.74	27.30	129.79	129.92	27.30	29.74	29.41	29.60	29.60	29.41	31.93	22.72	14.10
(11)c	180.50	34.12	24.67	29.06	29.40	29.25	29.25	29.25	29.79	27.21	129.84	129.94	27.21	29.79	29.40	29.55	29.40	31.94	22.72	14.09
(12)c	180.35	34.18	24.72	29.11	29.45	29.25	29.25	29.59	29.25	29.79	27.21	129.83	129.83	27.21	29.74	29.25	29.30	31.94	22.72	14.09
(13)c	180.50	34.08	24.67	29.06	29.40	29.64	29.79	29.64	29.64	29.40	29.79	27.30	129.82	129.82	27.30	29.79	29.01	31.74	22.72	14.09
(14)c	180.45	34.12	24.82	29.11	29.40	29.69	29.69	29.69	29.69	29.69	29.40	29.69	27.25	129.82	129.82	27.52	29.38	31.48	22.50	14.09
(15)c	180.21	34.03	24.67	29.06	29.41	29.69	29.69	29.69	29.69	29.69	29.69	29.41	29.79	27.30	129.85	129.85	27.01	31.88	22.24	13.99

FOOTNOTES

a) Nomenclature refers to:- Chain Length:Degree of Unsaturation(Position of Unsaturation)*cis* Double Bond

TABLE 41

^{13}C NMR Chemical Shifts and Assignments of Monounsaturated *trans*-Alkenoic Acids

Acid[a]	Shift and Assignment(ppm)																			
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}
12:1(5)t	180.50	33.44	24.57	31.91	128.68	131.96	32.62	29.60	28.96	31.79	22.72	14.09	–	–	–	–	–	–	–	–
(6)t	180.51	33.98	24.18	29.06	32.21	129.44	131.10	32.62	29.26	31.98	22.70	14.10	–	–	–	–	–	–	–	–
(7)t	180.11	34.13	24.62	28.67	29.30	32.37	129.94	130.67	32.37	31.94	22.28	13.99	–	–	–	–	–	–	–	–
(8)t	180.25	34.13	24.67	29.01	28.87	29.35	32.47	130.27	130.27	34.71	22.73	13.64	–	–	–	–	–	–	–	–
(9)t	180.50	34.18	24.67	29.11	29.40	28.91	29.60	32.68	129.73	131.90	25.67	14.19	–	–	–	–	–	–	–	–
14:1(5)t	180.32	33.49	24.51	31.91	128.73	131.94	32.57	29.64	29.41	29.60	29.41	31.94	22.67	14.09	–	–	–	–	–	–
(6)t	180.25	34.03	24.18	29.01	32.18	129.45	131.01	32.62	29.64	29.25	29.25	31.94	22.72	14.09	–	–	–	–	–	–
(7)t	180.35	34.13	24.62	28.62	29.01	32.37	129.84	130.67	32.62	29.64	28.96	31.76	22.72	14.10	–	–	–	–	–	–
(8)t	180.30	34.13	24.72	29.06	28.91	29.40	32.62	130.03	130.52	32.62	29.40	31.50	22.62	14.09	–	–	–	–	–	–
(9)t	180.50	34.13	24.72	29.06	29.06	28.96	29.69	32.62	130.18	130.38	32.33	31.84	22.28	13.99	–	–	–	–	–	–
(10)t	180.11	34.28	24.82	29.11	29.30	29.30	29.16	29.69	32.62	130.47	130.13	34.71	22.72	13.65	–	–	–	–	–	–
(11)t	180.11	34.13	34.67	29.06	29.40	29.64	29.54	29.21	29.69	32.62	129.70	131.91	25.62	14.14	–	–	–	–	–	–
16:1(5)t	180.48	33.44	24.58	31.90	128.67	131.94	32.62	29.74	29.41	29.69	29.69	29.74	29.41	31.93	22.76	14.09	–	–	–	–
(6)t	180.25	33.98	24.19	29.01	32.18	129.49	131.16	32.62	29.69	29.26	29.69	29.69	29.40	31.98	22.72	14.10	–	–	–	–
(7)t	180.52	34.09	24.67	28.67	29.26	32.37	129.90	130.68	32.62	29.69	29.40	29.60	29.40	31.84	22.72	14.09	–	–	–	–
(8)t	180.50	34.13	24.67	29.06	28.86	29.40	32.52	130.04	130.52	32.62	29.74	29.21	29.21	31.94	22.72	14.09	–	–	–	–
(9)t	180.16	34.08	24.67	29.06	29.41	28.91	29.60	32.52	130.18	130.47	32.62	29.60	28.91	31.75	22.67	14.09	–	–	–	–
(10)t	180.21	34.13	24.67	29.21	29.40	29.40	29.21	29.74	32.62	130.23	130.38	32.62	29.40	31.50	22.62	14.10	–	–	–	–
(11)t	180.25	34.18	24.72	29.11	29.45	29.45	29.50	29.25	29.69	32.62	130.33	130.33	32.33	31.98	22.23	13.99	–	–	–	–
(12)t	180.38	34.13	24.72	29.11	29.45	29.45	29.64	29.45	29.16	29.64	32.62	130.57	130.09	34.72	22.77	13.66	–	–	–	–
(13)t	180.50	34.13	24.77	29.06	29.41	29.64	29.69	29.69	29.41	29.26	29.74	32.62	129.70	131.89	25.67	14.14	–	–	–	–
18:1(6)t	180.35	33.98	24.18	29.01	32.18	129.45	131.11	32.62	29.69	29.25	29.69	29.69	29.69	29.69	29.40	31.98	22.72	14.10	–	–
(7)t	180.38	34.13	24.62	28.67	29.25	32.37	129.89	130.67	32.62	29.69	29.40	29.69	29.69	29.69	29.40	31.98	22.72	14.09	–	–
(8)t	180.68	34.13	24.67	29.01	28.91	29.35	32.52	130.04	130.52	32.62	29.69	29.25	29.69	29.69	29.41	31.94	22.67	14.09	–	–
(9)t	180.50	34.13	24.67	29.06	29.30	28.91	29.64	32.52	130.19	130.48	32.62	29.96	29.30	29.60	29.40	31.93	22.72	14.09	–	–
(10)t	180.21	34.18	24.72	29.30	29.40	29.30	29.30	29.74	32.67	130.23	130.38	32.67	29.76	29.30	29.30	31.98	22.67	14.10	–	–
(11)t	180.47	34.03	24.62	29.11	29.39	29.39	29.39	29.20	29.64	32.62	130.28	130.37	32.62	29.64	28.91	31.76	22.67	14.09	–	–
(12)t	180.50	34.12	24.67	29.21	29.40	29.40	29.64	29.64	29.21	29.64	32.61	130.33	130.33	32.61	29.40	31.45	22.62	14.09	–	–
(13)t	180.11	34.19	24.72	29.21	29.59	29.59	29.59	29.59	29.59	29.21	29.59	32.61	130.33	130.33	32.33	31.84	22.23	13.99	–	–
(14)t	180.25	34.18	24.67	29.11	29.34	29.60	29.69	29.69	29.69	29.60	29.21	29.69	32.62	130.56	130.07	34.71	22.73	13.65	–	–
20:1(9)t	180.50	31.08	24.72	29.06	29.34	28.96	29.69	32.68	130.23	130.51	32.67	29.69	29.34	29.69	29.69	29.69	29.34	31.98	22.70	14.10
(10)t	180.49	34.12	24.67	29.21	29.41	29.30	29.21	29.74	32.66	130.23	130.37	32.66	29.74	29.21	29.41	29.30	29.41	31.94	22.72	14.09
(11)t	180.11	34.13	24.67	29.11	29.40	29.25	29.50	29.25	29.69	32.62	130.28	130.38	32.62	29.69	29.25	29.50	29.40	31.93	22.72	14.09
(12)t	180.06	34.23	24.72	29.21	29.49	29.21	29.21	29.55	29.21	29.69	32.62	130.33	130.33	32.62	29.69	29.21	29.21	31.93	22.72	14.09
(13)t	180.21	34.18	24.67	29.06	29.41	29.60	29.74	29.74	29.74	29.60	29.74	32.62	130.32	130.32	32.62	29.74	28.91	31.76	22.65	14.09
(14)t	180.16	34.08	24.62	29.25	29.40	29.54	29.69	29.69	29.69	29.54	29.25	29.69	32.67	130.30	130.30	32.67	29.40	31.90	22.67	14.10
(15)t	180.35	34.12	24.67	29.11	29.25	29.55	29.74	29.74	29.74	29.74	29.54	29.25	29.74	32.62	130.38	130.38	32.38	31.98	22.28	13.99

FOOTNOTES

a) Nomenclature refers to:- Chain Length:Degree of Unsaturation(Position of Unsaturation)*trans* Double Bond

In cases where two signals are observed, they are assigned on the basis of previously recorded values obtained from acids produced biosynthetically from ^{13}C labelled acetate.[245] Accordingly, the shift at higher field is assigned to the carbon nearest the carboxyl head group. The 12:1(8), 14:1(10), 16:1(12) and 18:1(14) acids, i.e. (n-4) acids are exceptions to this general trend where, because of the influence of the methyl group, the allocation of chemical shifts to these unsaturated carbons are reversed.

The chemical shifts of unsaturated carbons depend on the position of unsaturation along the alkyl chain. The salient feature is that the two absorptions shift in opposite directions, roughly doubling the nonequivalence as the unsaturated bond approaches the carboxyl group (and, to a lesser extent, the methyl group). The average chemical shifts induced by the carboxyl and methyl groups on unsaturated carbons in positional isomers of acetylenic and alkenoic acids are summarised in Table 42.

In view of the widely adopted formalism for explaining shifts in hydrocarbons and related molecules, which is based on a sum of, at most, four empirical terms attributed to α, β, γ and δ substituents[128] (see Part Two, Section One, 1) this nonequivalence is surprising. It has been reported that similar nonequivalences are observed for the methyl esters, alcohols and triacylglycerols.[245] Thus, although shifts for unsaturated carbons are apparently relatively insensitive to changes in headgroup, the observed nonequivalences are clearly linked to the C-1 functional group. This has been demonstrated by the absence of nonequivalence in parent alkenes.

TABLE 42.

Chemical Shift (ppm) Induced in Unsaturated Carbon Atoms in Acetylenic, *cis*-Alkenoic and *trans*-Alkenoic Acids by Carboxyl and Methyl Groups

$$CH_3(CH_2)_nC_b[\,]C_a(CH_2)_mCO_2H$$

Position of Unsaturation	Induced Shift (ppm)								
	Acetylenic Acids (80.19 ppm)			*cis*-Alkenoic Acids (129.82 ppm)			*trans*-Alkenoic Acids (130.32 ppm)		
	C_a	C_b	C_a+C_b	C_a	C_b	C_a+C_b	C_a	C_b	C_a+C_b
5-	-1.553	+1.350	2.903	-1.703	+1.523	3.226	-1.650	+1.610	3.260
6-	-0.804	+0.659	1.463	-0.877	+0.730	1.607	-0.873	+0.786	1.659
7-	-0.463	+0.317	0.780	-0.448	+0.390	0.838	-0.430	+0.348	0.778
8-	-0.253	+0.251	0.504	-0.236	+0.223	0.459	-0.285	+0.202	0.487
9-	-0.150	+0.143	0.293	-0.127	-0.166	0.293	-0.150	+0.155	0.305
10-	-0.062	+0.122	0.146	-0.034	+0.117	0.151	-0.091	+0.055	0.146
11-	-0.024	+0.025	0.050	-0.010	+0.065	0.075	-0.046	+0.053	0.099
(n-3)	-0.630	+1.356	1.986	-0.520	+1.628	2.148	-0.630	+1.580	2.210
(n-4)	+0.171	-0.170	0.341	+0.230	-0.270	0.500	+0.236	-0.251	0.487

Considering the distances involved, and the relatively slow decrease in nonequivalence with bond position, σ inductive effects can be ruled out as a mechanism of transmission. Similarly, distance and local symmetry of the site make differential solvent effects unlikely. Anisotropic effects associated with the headgroups can be ruled out even in the 5-isomers as the chemical shift between C-5 and C-6 carbons in saturated acids is less than 0.2 ppm. Steric explanations based on molecular association are also unlikely because of the similarity of the effect in acids, esters and triacylglycerols which are expected to have vastly different associative properties.

With most common sources of chemical shift perturbation eliminated, Batchelor et al. have proposed that the observed nonequivalences of the ^{13}C chemical shifts of unsaturated carbons in monoenoic fatty acids are caused by electric fields from the headgroup.[245,246] Very briefly, the shifts can be interpreted in terms of changes in electron density at the various carbons as a result of polarisation of the bonds by the electric field. Such changes, δ_q, can be estimated as follows

$$\delta_q = \Sigma k_{11} E_l / e\ell \qquad (1)$$

where the sum is over all the bonds in an atom, E_l is the field resolved parallel to the bond, ℓ the length of the bond, k_{11} the empirical longitudinal bond polarisability, and e the electronic charge. The shift is given by

$$\delta_{e\ell} = (\delta/e)\delta_q \qquad (2)$$

where δ/e is the shift/electron charge for the carbon in question. For isolated unsaturated bonds, the effect of such polarisations is to cause equal and opposite shifts of the two unsaturated carbon signals, the unsaturated carbon nearer the headgroup shifting upfield, and the other

downfield. Table 42 demonstrates that this is generally the case.

Electric field effects are experienced by all carbons although in general, linear electric field shifts of methylene groups are very small because of the nearly equal polarisabilities of C-C and C-H bonds. Nevertheless, although small, such effects can in part explain the slightly lower chemical shift than expected (29.74 ppm) for the alkyl chain carbon absorptions in the shorter chain length saturated acids. The unsaturated carbons have high linear electric field shifts because a highly polarisable unsaturated bond is adjacent to C-C and C-H bonds of much lower polarisability. This results in a large accumulation or depletion of charge on the unsaturated carbon as a result of an electric field. The situation is somewhat different in the case of substituent effects resulting from the methyl group as, in addition to electric field effects, other sources of chemical shift perturbation such as steric and inductive effects also contribute to the induced shifts in an additive manner.

Finally, comparison of the shifts of unsaturated carbons in alkenoic and acetylenic acids indicate that there is approximately the same sensitivity to electric field effects for double and triple bonds. On the basis of polarisabilities alone, it would be expected that the nonequivalence would be 1.5 times greater in acetylenic acids as the longitudinal polarisability (ℓ_{11}) of a triple bond is greater than that of a double bond (3.5×10^{-24} vs. 2.8×10^{-24}). However, differences in sensitivity to electron density for various carbons must also be taken into account. Thus, a greater longitudinal polarisability is offset by a lower δ/e because of the linear symmetry of the -C≡C- group, resulting in similar electric field shifts for olefinic and acetylenic carbons.

A more detailed account of the electric field theory may be obtained in the literature.[245,246] It is sufficient to know for purposes required here that the phenomenon produces chemical shifts in the unsaturated carbon atoms which unambiguously allows the identification of position of unsaturation except in those few compounds where only one unsaturated carbon signal is observed.

From the data in Table 42, it is possible to quantify the effect of the carboxyl and methyl groups on unsaturated carbon atoms. These values can be used to calculate the chemical shift of unsaturated carbon atoms in any acetylenic or alkenoic acid. The use of this information for the calculation of chemical shifts of unsaturated carbons in monounsaturated acids is demonstrated in Table 43.

TABLE 43

Calculated, Observed and Literature ^{13}C Chemical Shifts of Unsaturated Carbons in some Monounsaturated Acids

Acid	Carbon	Induced Shift COOH CH_3		Chemical Shift (ppm) Calculated	Observed	Literature
14:1(7)a	C-7	80.19 - 0.463 + 0.000	=	79.727	79.72	79.72[255]
	C-8	80.19 + 0.317 - 0.000	=	80.507	80.50	80.52
14:1(10)a/(n-4)	C-10	80.19 - 0.062 + 0.171	=	80.299	80.30	-
	C-11	80.19 + 0.122 - 0.170	=	80.142	80.11	-
18:1(8)c	C-8	129.82 - 0.253 + 0.000	=	129.567	129.60	129.65[249]
	C-9	129.82 + 0.251 - 0.000	=	130.071	130.04	130.17
16:1(5)c	C-5	129.82 - 1.703 + 0.000	=	128.117	128.12	-
	C-6	129.82 + 1.523 - 0.000	=	131.343	131.34	-
18:1(6)t	C-6	130.32 - 0.873 + 0.000	=	129.447	129.45	129.49[249]
	C-7	130.32 + 0.786 + 0.000	=	131.106	131.11	131.13
14:1(10)t/(n-4)	C-10	130.32 - 0.091 + 0.236	=	130.465	130.47	-
	C-11	130.32 + 0.055 - 0.251	=	130.124	130.32	-

Generally, the induced shifts in the acetylenic acids are in good

agreement with literature values, and, with the odd exception, correlation is within ±0.05 ppm.[255] Values for *cis* and *trans* acids differ slightly from those quoted in the literature although they follow the same general trends.[249] This difference arises principally because the standard value for an isolated olefinic carbon differs by about 0.08 ppm with values quoted in the literature (129.90 ppm/*cis* and 130.40 ppm/*trans*).[249] Once corrected for this however, literature values compare favourably with observed shifts. In comparing literature and recorded data, whereas some ambiguity between chemical shifts may arise, the combined induced shift (C_a+C_b) can be very indicative of positional isomerism. It is further considered, that values quoted for induced shifts because of position of unsaturation in relation to functional groups in *trans* acids, are more reliable. Values quoted in the literature are based on *trans*-octadecenoic acids only[249] whereas values quoted in Table 42 are based on at least three examples of each acid.

Propargylic and Allylic Carbon Atoms

Propargylic and allylic carbon atoms are strongly influenced by the adjacent unsaturated system. In the absence of any influence from carboxyl and methyl groups, the chemical shifts of these carbons in acetylenic, *cis* alkenoic and *trans* alkenoic acids are on average 18.747, 27.303 and 32.616 ppm respectively. These shifts correspond to upfield shifts of about 11.0 and 2.5 ppm and a downfield shift of 2.9 ppm relative to methylene absorptions in a fully saturated acid (29.74 ppm). Furthermore, in several cases, two signals are observed where propargylic and allylic carbons are affected by deshielding arising from carboxyl or terminal methyl groups. These induced shifts are summarised in Table 44. The nonequivalences are considerably less compared to those

TABLE 44

Chemical Shift Induced in Propargylic and Allylic Carbon Atoms of Acetylenic, *cis*-Alkenoic and *trans*-Alkenioc Acids by Carboxyl and Methyl Groups

$CH_3(CH_2)_nC_b[\,]C_a(CH_2)_mCOOH$

Position of Unsaturation	Induced Shift (ppm)					
	Acetylenic Acids (18.747 ppm)		*cis*-Alkenoic Acids (27.303 ppm)		*trans*-Alkenoic Acids (32.618 ppm)	
	C_a-1	C_b+1	C_a-1	C_b+1	C_a-1	C_b+1
5-	-0.437	-	-0.790	-	-0.713	-
6-	-0.220	-	-0.439	-	-0.439	-
7-	-0.075	-	-0.247	-	-0.244	-
8-	0.000	-	0.000	-	-0.098	-
9-	-	-	-	-	-0.037	-
(n-3)	-	-6.257	-	-6.718	-	-6.967
(n-4)	-	+2.072	-	+2.414	-	+2.096
(n-5)	-	-0.269	-	-0.309	-	-0.273
(n-6)	-	0.000	-	0.000	-	0.000

experienced by unsaturated carbon atoms in all but the (n-3) and (n-4) isomers where it is dramatic.

These nonequivalences have been explained by Batchelor et al. in terms of the electric field theory.[245,246] The comparatively small differences are consistent with the fact that linear electric field shifts of methylene groups are small because of the nearly equal polarisabilities of C-C and C-H bonds.

The shift of propargylic carbon atoms may be explained partially, but not entirely, by the diamagnetic anisotropy of the triple bond. Additionally, there is an increase in local diamagnetic shielding. This results from an increase in electron density at the propargylic carbons which tends to expand the 2p orbitals.

The shifts exhibited by *cis* and *trans* allylic carbons may be explained primarily in terms of steric interactions. The allylic carbons in *cis* acids have a strong steric interaction with each other as a result of the *cis* configuration and subsequently absorb 5.3 ppm upfield of the corresponding *trans* acids which exhibit no such interaction.

Long Range Substituent Effects

Deshielding induced by an unsaturated bond is not confined to the adjacent (propargylic and allylic) carbon atoms, but shows itself at a reduced level for at least 5 carbon atoms. These effects are difficult to quantify in long chain acids because of the poor resolution of the methylene carbon signals. In shorter chain acids, as substituent effects are additive the deshielding induced by the carboxyl and methyl groups must also be considered. The shifts summarised in Table 45 are representative of the long range deshielding induced by unsaturated

bonds. These are consistent with literature values to within ±0.045 ppm.

TABLE 45

Long Range Deshielding Effects on the $(CH_2)_n$ Chemical Shift (29.741 ppm) of Isolated Unsaturated Bonds

Bond	Substituent Carbon and Induced Shift (ppm)					
	α	β	γ	δ	ε	ζ
Acetylenic	-10.971	-0.516	-0.829	-0.507	-0.200	-0.098
cis	-2.470	-0.049	-0.418	-0.160	-0.098	0.000
trans	+2.887	-0.098	-0.512	-0.196	-0.098	0.000

Influence of Unsaturated Carbons on the Carboxyl and Terminal Methyl Groups

In most acids, the unsaturated carbons exert a considerable influence on the carbons at particularly the methyl end but also, at the carboxyl end of the molecule. These induced shifts for acetylenic and alkenoic acids are summarised in Tables 46, 47 and 48. These are consistent with literature values although differences can occur between values of the carboxyl end as a result of solvent/solute concentration effects. In respect of values for the methyl end, agreement extends to literature values for the methyl alkenoates.[250,251]

The chemical shift of the carboxyl carbon atom of the acids falls between 180.0 and 180.6 ppm but does not exhibit any apparent trend with position of unsaturation in the 5- isomer onwards. Gunstone et al. however, report significant differences where unsaturation is 2-, 3- and 4- to the carboxyl. In such cases, *cis* acids exhibit mean shifts of 166.53, 178.52 and 180.04 ppm and acetylenic acids mean shifts of 158.40, 175.60 and 178.70 ppm. The effects of unsaturation on neighbouring carbons when the unsaturated bond is (n-1), (n-2) or 2- to 4- have also been characterised by Gunstone et al..[249,255]

TABLE 46

Chemical Shifts (ppm) Induced by the Triple Bond on Carbons at the Carbonyl and Methyl Ends of Monounsaturated Acetylenic Acids

Carbon	Sat.[a]	Average Induced Shift (ppm) Position of Triple Bond Relative to –COOH			
		5–	6–	7–	8–
C-2	34.129	-0.997	-0.487	-0.116	-0.082
C-3	24.671	-0.605	-0.372	-0.442	-0.124
C-4	29.059	-10.752	-0.537	-0.773	-0.457
C-5	29.400	+49.236	-10.873	-0.574	-0.780
C-6	29.595	+51.952	+49.781	-10.922	-0.432

		Position of Triple Bond Relative to $-CH_3$					
		(n-3)	(n-4)	(n-5)	(n-6)	(n-7)	(n-8)
(n-1)	14.090	+0.318	-0.585	-0.444	-0.097	-	-
(n-2)	22.699	-10.202	-0.125	-0.705	-0.466	-0.090	-
(n-3)	31.935	+49.600	-11.116	-0.591	-0.745	-0.438	-0.110
(n-4)	29.400	+50.156	+50.610	-10.929	-0.536	-0.731	-0.513

TABLE 47

Chemical Shifts (ppm) Induced by *cis* Double Bonds on Carbons at the Carbonyl and Methyl Ends of Monounsaturated Alkenoic Acids

Carbon	Sat.[a]	Average Induced Shift (ppm) Position of Double Bond Relative to –COOH			
		5–	6–	7–	8–
C-2	34.129	-0.637	-0.116	-0.046	-
C-3	24.671	-0.077	-0.369	0.000	-
C-4	29.059	-2.545	-0.030	-0.267	-0.120
C-5	29.400	+98.720	-2.536	+0.012	-0.436
C-6	29.595	+101.943	+99.557	-2.367	-0.044

		Position of Double Bond Relative to $-CH_3$					
		(n-3)	(n-4)	(n-5)	(n-6)	(n-7)	(n-8)
(n-1)	14.090	+0.307	-0.291	-0.097	-	-	-
(n-2)	22.699	-2.094	-0.196	-0.384	-0.200	-	-
(n-3)	31.935	+99.513	-2.281	-0.015	-0.446	-0.159	-
(n-4)	29.400	+99.900	+100.610	-2.406	-0.050	-0.352	-0.086

TABLE 48

Chemical Shifts (ppm) Induced by *trans* Double Bonds on Carbons at the Carbonyl and Methyl Ends of Monounsaturated Alkenoic Acids

Carbon	Sat.[a]	Average Induced Shift (ppm) Position of Double Bond Relative to –COOH			
		5–	6–	7–	8–
C-2	34.129	-0.692	-0.146	-0.012	-
C-3	24.671	-0.118	-0.488	-0.049	-
C-4	29.059	+2.966	-0.049	-0.439	-0.025
C-5	29.400	+99.291	+2.779	-0.109	-0.488
C-6	29.595	+102.544	+100.049	+2.974	-0.025

		Position of Double Bond Relative to $-CH_3$					
		(n-3)	(n-4)	(n-5)	(n-6)	(n-7)	(n-8)
(n-1)	14.090	+0.049	-0.440	-0.097	-	-	-
(n-2)	22.699	+2.952	-0.030	-0.437	-0.097	-	-
(n-3)	31.935	+95.957	+2.800	-0.097	-0.455	-0.174	-
(n-4)	29.400	+100.297	+100.677	+2.945	-0.035	-0.469	-0.146

GENERAL FOOTNOTES

a) Corresponds to usual chemical shift based on saturated acid (see Table 38)

Monounsaturated acids where unsaturation is (n-3) to (n-6) and 5- to 8- may be readily identified from the shifts induced in easily assigned signals at either end of the molecule. In conjunction with the observations of Gunstone et al., it may be concluded that such induced shifts facilitate the identification of monounsaturated acids where the position of unsaturation is 2- to 8- and (n-1) to (n-8).

iv) Polyunsaturated Fatty Acids

^{13}C Chemical shifts of PUFAs recorded are summarised in Table 49 and the ^{13}C spectrum of Linelaidic Acid (18:2(9,12)t,t) is illustrated in Figure 37. These limited number of spectra, exhibit characteristic differences on the basis of configuration, position and degree of unsaturation.

The spectra of dienoic acids exhibit four absorptions corresponding to unsaturated carbon atoms. In linoleic acid, these are at 130.04 (C-9), 128.13 (C-10), 127.94 (C-12) and 130.23 ppm (C-13). These signals in the corresponding *trans* isomer are slightly downfield at 130.86, 128.72, 128.52 and 131.06 ppm. The chemical shifts of the methylene group between the double bond are 25.65 ppm (*cis*) and 35.64 ppm (*trans*) and the chemical shifts of the remaining allylic carbons are 27.26 ppm (*cis*) and 32.57 ppm (*trans*).

The remaining carbons exhibit similar chemical shifts to those in saturated and monounsaturated acids. Once again however, these shifts may be influenced in a characteristic manner by the proximity of the double bond. Effectively, each olefinic centre is influenced by the acid group, the terminal methyl and other unsaturated bonds in an additive manner. These effects for a number of PUFAs have been reported and evaluated by Gunstone et al.[249,255] and have been explained in terms of

TABLE 49

^{13}C NMR Chemical Shifts and Assignments of Polyunsaturated Fatty Acids

Acid[a]	Shift and Assignment (ppm)																	
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}
18:2(9,12)	180.45	34.13	24.67	29.11	29.11	29.11	29.60	27.26	130.04	128.13	25.65	127.94	130.23	27.26	29.35	31.55	22.57	14.09
18:2(9,12)[b]	180.45	34.13	24.67	29.06	29.06	29.25	29.45	32.57	130.86	128.72	35.64	128.52	131.06	35.57	29.25	31.45	22.57	14.09
18:3(6,9,12)	180.45	34.02	24.31	29.06	26.94	129.58	128.35	25.64	128.15	128.50	25.64	127.71	130.43	27.26	29.40	31.55	22.62	14.09
18:3(9,12,15)	180.45	34.13	24.67	29.11	29.11	29.11	29.60	27.26	130.18	127.74	25.60	128.28	128.28	29.55	127.16	131.94	20.58	14.29
18:4(4,8,12,15)	179.68	33.93	24.28	127.01	131.99	28.96	26.82	128.18	127.89	25.60	25.60	128.23	128.23	25.60	127.89	129.55	20.58	14.29
20:4(5,8,11,14)[c]	180.35	34.08	24.61	26.85	128.95	128.95	25.73	128.24	128.24	25.73	127.96	128.65	25.73	127.61	130.51	27.30	29.40	31.55

FOOTNOTES

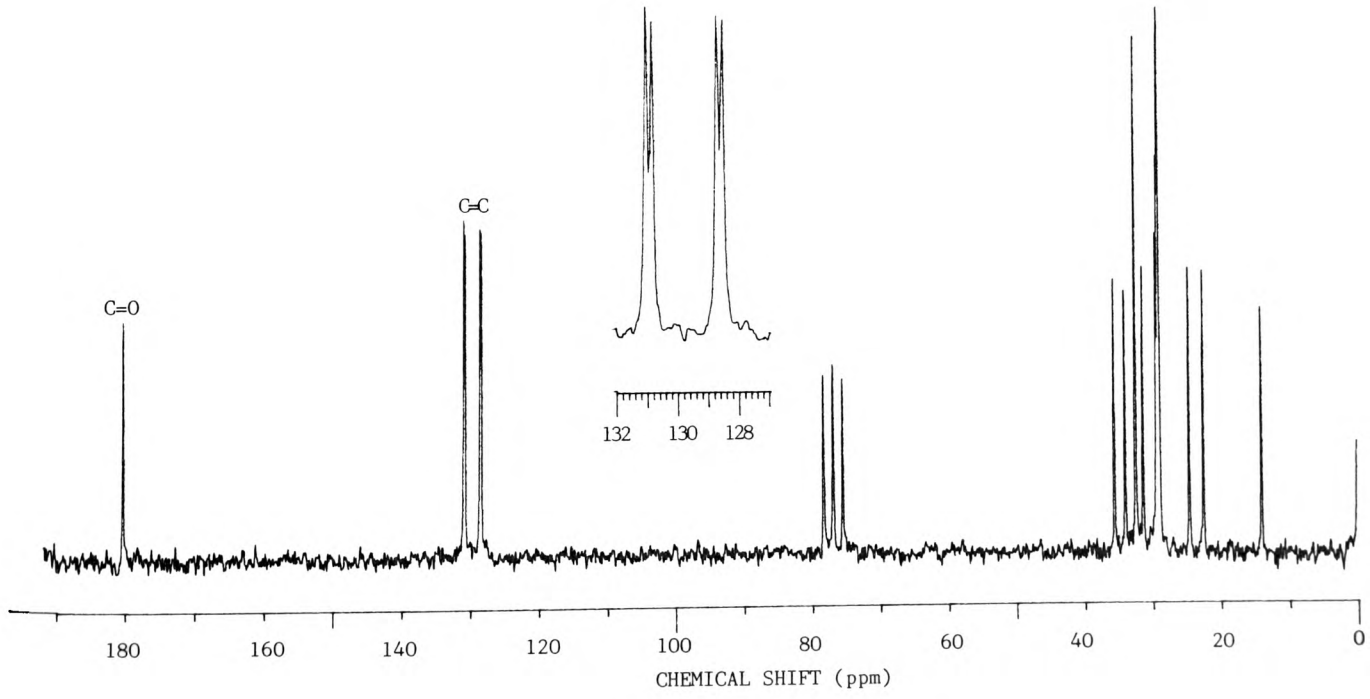
a) Unless stated otherwise, configuration of the double bonds are *cis*.

b) Double bond configuration is *trans*.

c) C_{19} 22.67, C_{20} 14.09.

FIGURE 37 ^{13}C NMR Spectrum of Linelaidic (*trans,trans*-9,12-Octadecadienoic) Acid

$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$



electric field theory by Batchelor et al.[245,246] In the case of PUFAs, electric field contributions are considerably more complicated. They arise both from the dipolar headgroup and from polar bonds associated with other double bonds. The headgroup electric field effects are apparently very little different from those observed in monounsaturated chains. Substituent shifts induced by double bonds however, contain contributions because of electric fields and steric shifts.

As with the ^{1}H spectra, shifts induced in the absorption of the terminal methyl group by an unsaturated bond are the same as those induced in monounsaturated acids. The methyl carbon in acids involving (n-3) unsaturation absorb downfield of the normal chemical shift at 14.29 ppm. Furthermore, a significant upfield shift is induced in the carboxyl carbon absorption of moroctic acid (179.68 ppm) by the unsaturated bonds.

The ^{13}C Spectra of a number of PUFAs as the methyl esters have previously been reported in the literature.[249,250,251,255]

d) The Analysis of Fatty Acids by ^{17}O NMR Spectroscopy

The use of ^{17}O NMR spectroscopy as a structural probe has been comprehensively reviewed by Amour[256] and Klempler.[257] The ^{17}O nucleus has a spin I=5/2 and a quadrupole moment Q=-2.6 x 10^{-26} cm^2. Thus the ^{17}O nucleus possesses an ellipsoidal charge distribution and is capable of interacting with electric field gradients induced by the electrostatic environment. In the liquid state, this time dependent coupling is a very efficient mechanism of relaxation and spin lattice relaxation times (T_1) for ^{17}O are in general in the order of milliseconds.

The low natural abundance of this isotope (0.037%), however, and the

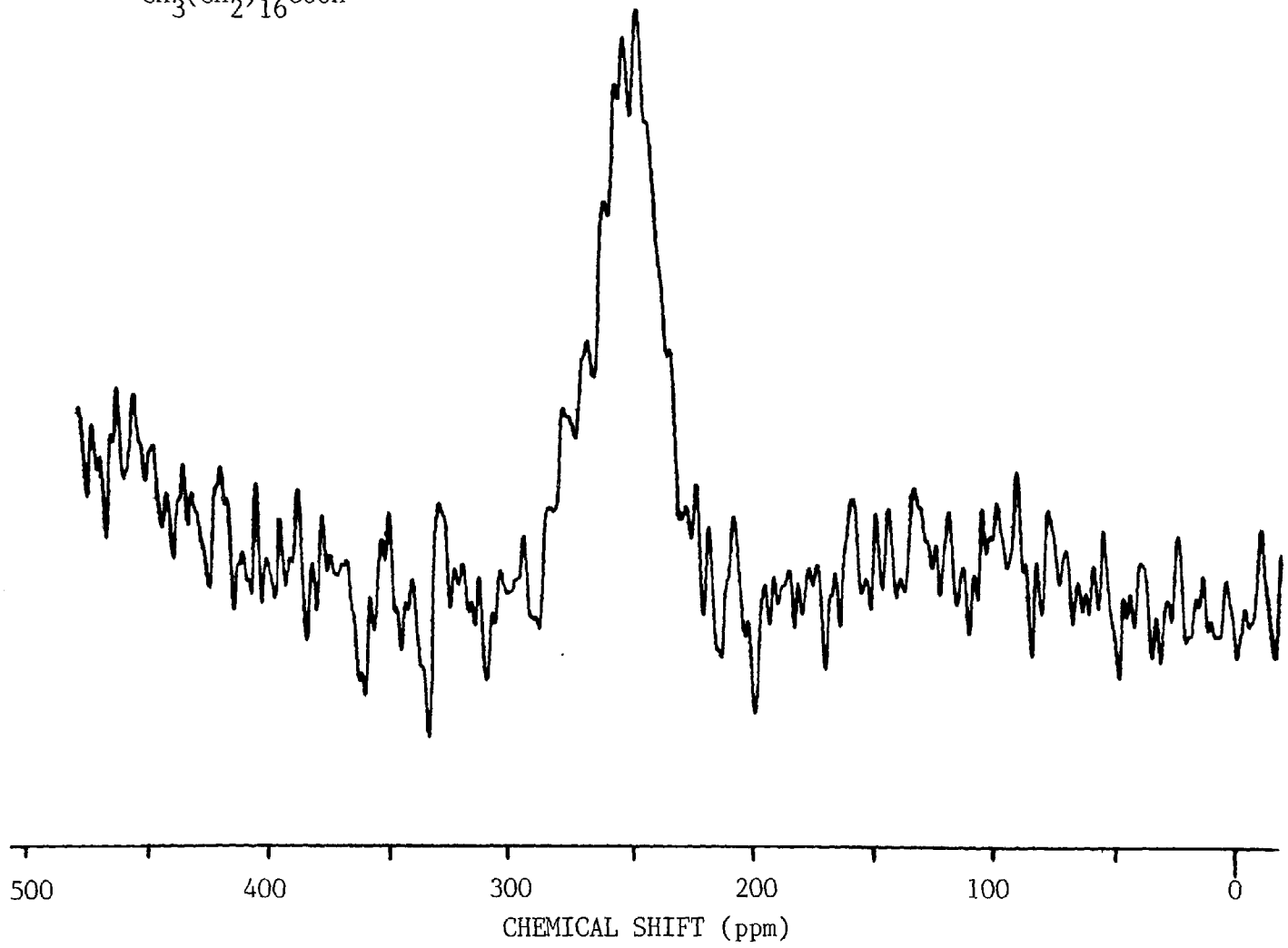
existence of an electric quadrupole moment has the general effect of broadening the spectral lines because of efficient quadrupolar relaxation. Consequently these limitations have in the past confined the use of ^{17}O NMR spectroscopy to the study of low molecular weight compounds.

Nevertheless, ^{17}O NMR spectroscopy of saturated solutions of fatty acids in $CDCl_3$ resulted in a single absorption ~252 ppm downfield of D_2O as an external reference. By comparison, in the methyl ester derivatives, two signals were observed at ~358 ppm (C=O) and ~141 ppm ($-OCH_3$). The single absorption in the acid spectra is not surprising as the rate of proton exchange, and the dimeric structure, leads only to one time-averaged ^{17}O NMR signal.

Figure 38 is typical of the spectra obtained. Due to the low natural abundance of the ^{17}O nucleus, long accumulation times are necessary to obtain a spectrum even of this quality ($>10^6$ pulses). The synthesis and use of ^{17}O enriched fatty acids would be beneficial in combination with extensive signal accumulation.

FIGURE 38 ^{17}O NMR Spectrum of Octadecanoic (Stearic) Acid

$CH_3(CH_2)_{16}COOH$



SECTION FOUR

SUMMARY AND CONCLUSIONS

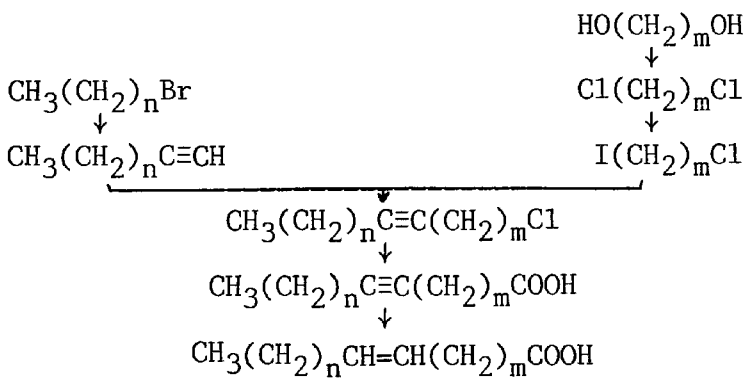
The process of hydrogenation can dramatically alter the fatty acid composition of an oil resulting in the formation of among other components, geometrical and positional isomers of monounsaturated fatty acids.

The view that there may be hazards associated with the altered fatty acid components of industrially-hydrogenated oils has occasionally been expressed. There is statistical evidence to suggest that people who had died of IHD consumed a higher amount of margarines based on commercially hydrogenated oils, particularly HMO, relative to RAF than did people who had died from unrelated causes.

Prior to an investigation into the levels of positional and geometrical fatty acid isomers in adipose tissue and dietary fats, the synthesis of a series of these acids as standards, and their characterisation was necessary.

Although the synthesis of such acids have been previously reported, the emphasis has been on the synthesis of C_{18} acids. In the context of the UK diet, because of the diverse nature of oils and fats used, the fatty acid profile in addition to 18:1 acids consists of C_{12}-C_{22} chain length monounsaturated fatty acids.

The general synthetic scheme employed is illustrated below:-



This acetylenic approach provided a convenient route for the synthesis of both geometrical and positional monounsaturated fatty acid isomers from common precursors.

1-Alkynes and α-chloro-ω-iodoalkanes may be condensed in any combination to give the desired chain length and position of unsaturation. When this condensation was performed via sodamide in liquid ammonia, the scheme was limited to the synthesis of acids where n>7 and m=5-9 but was extended somewhat when performed via methyllithium in dioxan. In general, yields were good for the synthesis of shorter chain length compounds and where the position of unsaturation was relatively central. Yields decreased with increasing chain length and migration of the unsaturated bond to the extremities of the resulting molecule. Addition of one or two carbon atoms to the resulting 1-chloroalkynes by traditional methods yielded the acetylenic acid.

For long-term storage, the acids were kept as acetylenic acids. These were converted as required to either the *cis*-alkenoic acids by catalytic hydrogenation over Lindlar's catalyst or the *trans*-alkenoic acid by reduction with lithium or sodium in liquid ammonia. A total of 74 *cis* and *trans* acids of C_{12}-C_{20} even chain lengths were synthesised by this general method. These acids, together with 10 commercially available monounsaturated acids were qualitatively characterised by chromatographic and spectroscopic methods.

Traditionally, the quantification of monounsaturated positional isomers has involved the GLC analysis of the ozonide cleavage products, after prior isolation on the basis of chain length, degree of unsaturation and configuration of unsaturation. Even with these procedures, a completely

accurate analysis of both double bond position and configuration is difficult. Furthermore, such procedures are time consuming and laborious and a method permitting the direct quantification of positional iosmers is desirable.

Reverse-phase HPLC on a C_{18}-bonded phase column and a water/acetonitrile solvent system was applied to the separation of the methyl ester derivatives. Separations were monitored by a UV variable wavelength detector set at 210 nm. Separation on the basis of chain length, degree and configuration of unsaturation is dramatic. Furthermore, the partial separation of positional isomers was achieved; a six component mixture partially resolving into five peaks.

The partial separation of positional isomers by capillary column GLC was also achieved but in view of previously reported separations, these were somewhat dissappointing. This was due in part to equipment limitations. The development and optimisation of the technique offers the most encouragement for the direct quantification of positional isomers on a relatively rapid basis.

NMR spectroscopy, particularly ^{13}C, unambiguously determined the geometry and position of a double bond within virtually every fatty acid. Assignment is based on the fact that functional groups within an acid influence the chemical shift of neighbouring carbons in a characteristic manner. Configuration of the double bond was determined by characteristic shifts in the olefinic and allylic carbon and proton signals as summarised overleaf:-

	Chemical Shift (ppm)			
	Olefinic		Allylic	
Acid	^{1}H	^{13}C	^{1}H	^{13}C
cis	5.32	129.80	2.01	27.62
trans	5.36	130.32	1.95	32.62

Positional assignment in ^{13}C was made on the basis of the two unsaturated carbons exhibiting characteristic chemical shifts for each positional isomer, and shifts induced by double bonds in easily assigned carbons at the ends of the molecule. These shifts allow identification of positional isomers in all acids except those few which exhibit only one signal for the unsaturated carbons.

Similarly, but to a lesser extent, shifts induced by the configuration and position of unsaturation in the 90 MHz ^{1}H spectra may be used to identify acids where the position of unsaturation is 5- to 7- in *cis* and *trans* acids, (n-3)-(n-6) in *cis* acids and (n-3)-(n-4) in *trans* acids. Generally however, ^{1}H NMR spectroscopy is not particularly useful as a diagnostic aid at 90 MHz. In view of data for 220 MHz spectra which report the identification of all the C_{18} monounsaturated acids except the 10-, 11- and 12- isomers, the use of 400 and 500 MHz instruments would be particularly useful.

^{17}O NMR spectra are difficult to obtain due in part to the isotope's low natural abundance and line broadening due to efficient quadrupolar relaxation. As such they are of little diagnostic value. Nevertheless, methyl ester derivatives exhibit two absorptions at ~358 ppm (C=O) and ~141 ppm ($-OCH_3$) relative to D_2O as an external standard when run as a saturated solution in $CDCl_3$. The acids, because of the rate of proton exchange and the dimeric structure, exhibit one time averaged signal at

~252 ppm.

Although NMR spectroscopy, particularly ^{13}C, may be employed to characterise fatty acids on an individual basis, the application of the technique to the determination of positional isomers in a lipid mixture is somewhat more limited. The determination of positional isomers in a simple standard mixture of C_{18} acids was readily achieved but determination is more difficult in a complex mixture. NMR specrtoscopy does however have certain advantages for the analysis of fatty acids in lipid mixtures in that it can be non-destructive, requires no derivatisation and is reasonably quantitative.

Several workers have applied NMR spectroscopy to the analysis of oils and fats. Johnson and Shoolery have applied ^{1}H NMR to the determination of total unsaturation of triacylglycerols and conclude that rapid non-destructive analysis of the degree of unsaturation in small samples of fats and oils may be regarded as routine.[237] ^{13}C NMR has been used to determine the extent of *trans* unsaturation in lipid mixtures. Such measurements were obtained by measuring the ratio of the *cis* and *trans* allylic carbon signals. It is reported that as long as adequate precautions are taken to ensure that nuclear Overhauser effects are suppressed, results compare favourably with traditional methods of determination of *trans* unsaturation.[202] ^{13}C NMR has also been used to profile several fats and oils[204] and for the qualitative analysis of several seed oils.[203]

With regard to future work, no one technique alone is at present satisfactory for the complete determination of positional isomerism in a complex fatty acid mixture. Traditional techniques have involved

analysis by ozonolysis and this requires the separation of *cis* and *trans* isomers prior to oxidative cleavage. This separatation has been carried out by argentation chromatography although overlap between components can occur. In a vegetable oil where monounsaturation is predominantly 18:1 for example, contamination is minimal. However, in a lipid sample such as HMO or adipose tissue where monounsaturation covers a range of chain lengths from C_{12}-C_{22}, difficulties can arise. Argentation chromatography seperates not only on the basis of the geometry of the double bond, but also chain length and degree of unsaturation, a considerable overlap of components can occur making interpretation of subsequent analyses extremely difficult.

Separation of components must first be achieved on the basis of chain length by either perparative HPLC or GLC and then degree of unsaturation by argentation chromatography. The monoene fractions could then be subjected to other forms of analysis such as ^{13}C NMR spectroscopy or capillary column GLC. As with traditional methods, these procedures are rather time consuming but one stage, that of ozonolysis, is eliminated.

The disadvantage of ^{13}C NMR spectroscopy to the analysis of lipid mixtures is its relative insensitivity compared to capillary column GLC. Furthermore, for quantitative analysis, care must be taken to ensure that nuclear Overhauser effects are suppressed.

Although many modern capillary columns are capable of partial resolution of positional isomers, no column is yet available that can resolve all *cis* and *trans* fatty acid isomers found in HF. The technique is limited by the currently available stationary phases and the main disadvantages to its application for a large scale case vs. control study are

relatively short column life and expense compared to packed columns. However, the use of the highly polar SP-2560 column, recently developed by Supelco holds particular promise.[209] Continuing development and optimisation of this technique should form the basis of future work in this area.

PART THREE

EXPERIMENTAL

1 General Notes

(1) Melting points were taken on a Gallenkamp melting point apparatus.

(2) Melting points and boiling points are all uncorrected.

(3) $^{1}H, ^{13}C$ and ^{17}O NMR spectra were recorded on a Jeol FX90Q FT spectrometer operating at 89.55, 22.49 and 12.11 MHz respectively.

(4) ^{1}H and ^{13}C chemical shifts are given in ppm downfield from TMS as an internal standard. ^{17}O Shifts were recorded relative to external D_2O.

(5) IR spectra were recorded on either a Perkin-Elmer 681 or 881 IR spectrometer interfaced with a Perkin-Elmer 3600 Data Station.

(6) HPLC were run on a Varian 5000 liquid chromatograph interfaced with a Vista 402 data station. The detector was a Varian UV-50 variable wavelength detector set at 210 nm.

(7) GLC were run on a Perkin-Elmer F17 gas chromatograph fitted with dual flame ionisation detectors and interfaced with a Perkin-Elmer 3600 Data Station.

2 Solvents and Reagents

Acetone. To GPR acetone (1.4l) was added a solution of silver nitrate in water (6g/40cm^3) and 1M Sodium hydroxide solution (40cm^3). The mixture was shaken (10 minutes), filtered, dried ($CaSO_4$) and distilled. The first 10% of the distillate was discarded and pure acetone (b.p. 56.2°C/760mm) thereafter collected and stored over Type 4A molecular sieve until required for use.

HPLC Grade Acetonitrile. LC grade acetonitrile was purified if necessary by elution through activated silica gel. Calcium hydride was then added in portions with stirring until hydrogen evolution ceased. The solvent was subsequently decanted from the solid and fractionally distilled (b.p. 81-82°C/760mm).

Carbon Disulphide. AnalaR grade was found to be suitable and was simply dried over anhydrous $MgSO_4$.

Chloroform (ethanol free). Chloroform (GPR) was purified by washing three times with an equal volume of water and allowed to stand over $CaCl_2$ (2 days). The material was distilled and the remaining ethanol removed as an azeotrope with the chloroform (b.p. 59.7°C/760mm). Pure chloroform was collected at 61.2°C/760mm, restabilised with purified methanol (2%v/v) and stored in a cool, dark, dry place until required.

Diethyl Ether (Ether). AnalaR grade was found to be suitable and was simply dried over sodium wire.

Dioxan. Dioxan (GPR) (1l) was refluxed with concentrated hydrochloric acid (14cm^3) and water (100cm^3) (8-12 hours) under nitrogen. After cooling, the solution was treated with excess potassium hydroxide

pellets with shaking. The aqueous layer was removed and residual water removed by storing over fresh potassium hydroxide pellets (24 hours). The decanted solvent was refluxed over excess sodium (6-12 hours) and finally distilled (b.p. 101.5°C/760mm. The purified dioxan was stored in darkness under nitrogen until required for use.

"Super Dry" Ethanol. Absolute ethanol (50-75cm^3) was added to dry, clean magnesium turnings (5g) and iodine (0.5g). The mixture was heated until the iodine disappeared and heating continued until the magnesium had been converted to its enolate complex. Absolute ethanol (900cm^3) was added and the mixture refluxed (1 hour). This was distilled (b.p. 78.5°C/760mm) directly into the storage vessel with the necessary precautions to exclude moisture. "Super dry" ethanol was stored over Type 4A molecular sieve in a well sealed container until required for use.

Ethyl Acetate. Ethyl acetate (500cm^3), acetic anhydride (50cm^3) and concentrated sulphuric acid (5 drops) were heated under reflux (4 hours) before distillation. The distillate was shaken with anhydrous potassium carbonate (10-20g), filtered and redistilled (b.p. 77°C/760mm).

Hexane. Technical grade hexane (67-70°C fraction from petroleum spirit) (500cm^3) was washed with successive amounts of concentrated sulphuric acid (50cm^3) until the acid layer became almost colourless. Residual acid was removed by washing with water and sodium bicarbonate solution (2%). The hexane was dried ($CaCl_2$) prior to simple distillation. The distillate boiling between 67-68°C/760mm was collected.

Methanol (anhydrous). This was prepared in accordance with the procedure described above for "super dry" ethanol, collecting only the middle third of the distillate (b.p. 64.4°C/760mm). This was stored over Type

4A molecular sieve until required for use.

Pentane. Olefine free AnalaR grade was further purified by simple distillation. The fraction boiling at constant temperature (ca. 35°C/760mm) was collected.

Petroleum Ether (40-60°). Technical grade petroleum ether (40-60°) was purified in accordance with the procedure described above for hexane.

Quinoline. Quinoline ($100cm^3$) was purified by refluxing over potassium hydroxide pellets (4 hours) and distilled under reduced pressure (b.p. 49°C/0.5mm).

Tetrahydrofuran. Commercial grade THF was passed through an alumina column ($50g/100cm^3$) and refluxed over lithium aluminium hydride (4 hours). The solvent was distilled (b.p. 65-66°C/760mm) and stored over calcium hydride, under nitrogen until required.

HPLC Grade Water. Deionised water was double distilled and subsequently passed through a Norganic^{TM} Water Purification System (Waters Associates/Millipore).

Acetyl Chloride. Fresh AnalaR grade material was simply distilled as required. The first 20% of distillate was discarded leaving a similar volume of residual material. The collected distillate boiled at 59°C/760mm.

Thionyl Chloride. Commercial purified thionyl chloride was further purified by simple distillation (b.p. 77°C/760mm).

3 Preparation of α,ω-Dichloroalkanes

1,5-Dichloropentane and 1,6-dichlorohexane were obtained from Aldrich. Other α,ω-dichloroalkanes were prepared from the corresponding α,ω-diols in accordance with the procedure described for 1,8-dichlorooctane. The α,ω-diols were obtained from Aldrich with the exception of 1,9-nonanediol which was purchased from Lancaster Synthesis.

1,8-Octanediol (200g/1.37moles), dry pyridine ($25cm^3$) and thionyl chloride (78.1g/0.658moles) were melted together. To the cooled stirred mixture, thionyl chloride (248g/2.084moles) was added at such a rate that the temperature remained at about 38°C. After the addition, the flask and contents were heated by steam for two hours. After cooling, ice and water were carefully added, and the dense, precipitated oil taken up in petroleum ether (40-60°). This was washed twice with concentrated sulphuric acid, once with sodium bicarbonate solution and finally water. Drying ($MgSO_4$) and solvent removal left the crude product which was distilled to give 1,8-dichlorooctane in good yield (239.6g/95.6%) b.p.56-59°/0.5mm. IR; CH_2Cl(def) 1285 cm^{-1}, C-Cl(st) 722 and 650 cm^{-1}: ^1H NMR; CH_2Cl 3.52 ppm (triplet J=6.84 Hz): ^{13}C NMR; CH_2Cl 45.37 ppm.

4 Preparation of α-Chloro-ω-iodoalkanes

1-Chloro-3-iodopropane was obtained from Aldrich. Other α-chloro-ω-iodoalkanes were prepared by reacting the α,ω-dichloroalkane with sodium iodide in dry acetone in accordance with the procedure described for 1-chloro-5-iodopentane. Commercial 1,5-dichloropentane and 1,6-dichlorohexane were purified by distillation prior to use. Sodium iodide (GPR) was obtained from BDH, used as supplied and stored in a desiccator.

1,5-Dichloropentane (112.8g/0.8moles) was added to a solution of sodium iodide (119.2g/0.8moles) in dry acetone ($500cm^3$) and refluxed, with stirring (6 hours). Water ($400cm^3$) was then added and the organic layer separated. The aqueous layer was extracted with ether and the two organic fractions combined. After washing (sodium thiosulphate solution followed by water) and drying ($MgSO_4$), the solvent was evaporated, and the residue fractionally distilled to give:

(a) unchanged dichloride (26.6g) b.p.40-44°C/2.5mm,

(b) 1-chloro-5-iodopentane (121.6g/65.4%) b.p.66-88°C/2.5mm,

(c) 1,5-di-iodopentane (23.4g) b.p.90-92°C/2.5mm.

IR; CH_2Cl(def) 1285 cm^{-1}, CH_2I(def) 1173 cm^{-1}, C-Cl(st) 722 and 650 cm^{-1}: ^1H NMR; CH_2Cl 3.54 ppm (triplet J=7.20 Hz), CH_2I 3.20 ppm (triplet J=7.24 Hz): ^{13}C NMR; CH_2Cl 44.73 ppm, CH_2I 6.40 ppm.

5 Preparation of 1-Alkynes

5.1 Sodamide

Ferric nitrate (0.1g) and clean sodium (1.0g) were added with stirring to liquid ammonia ($750cm^3$). When the blue colour had been discharged, an additional amount of sodium (22.0g/1.0moles total) was added slowly in small proportions (1-2 hours). After a further 30 minutes, sodium peroxide (0.3g) was added. After 2.5 hours, the mixture assumed a dull grey colour, and the conversion of sodium to sodamide was judged to be complete. The original volume of the reaction vessel was maintained throughout by the addition of fresh liquid ammonia.

5.2 Sodium acetylide

Acetylene was passed through concentrated sulphuric acid and sodalime to remove acetone and bubbled through the suspension of sodamide until a

uniformly black liquid was formed (4-5 hours). The flow of acetylene was continued during the preparation of 1-alkynes.

5.3 1-Alkyne

1-Alkynes other than 1-butyne were prepared by reacting a 1-bromoalkane with sodium acetylide in accordance with the procedure described for 1-nonyne. 1-Butyne was prepared in a similar manner but using commercial sodium acetylide in xylene (Aldrich) and used without isolation in the next stage. 1-Bromoalkanes were obtained from Aldrich and distilled prior to use.

Freshly distilled 1-bromoheptane (161g/0.9moles) in ether (100cm^3) was rapidly added with stirring to sodium acetylide (30-40 minutes). The flow of acetylene was discontinued and the reaction mixture allowed to stand at -55°C (4-5 hours). Ammonium chloride (8g) was added with stirring to decompose the excess of sodium acetylide, followed by ice (100g), and water (500cm^3) (CARE!). The organic layer was extracted with ether, washed (water), dried ($MgSO_4$) and the solvent removed to leave a residue which on fractional distillation yielded 1-nonyne (94.4g/84.6%) b.p.33.3°C/10mm. IR; ≡CH(st) 3311 cm^{-1}, C≡C(s) 2119 cm^{-1}: ^1H NMR; ≡CH 1.92 ppm (triplet J=2.63 Hz), $CH_2C\equiv$ 2.16 ppm, CH_3 0.88 ppm (distorted triplet): ^{13}C NMR; C≡CH 68.08 ppm, C≡CH 84.67 ppm, CH_3 14.10 ppm.

6 Preparation of 1-Chloroalkynes

6.1 Via Sodium in Liquid Ammonia

Condensation of the appropriate 1-alkyne and α-chloro-ω-iodoalkane with sodamide as the condensing agent gave the desired 1-chloroalkyne in accordance with the procedure described for 1-chloro-7-pentadecyne.

Sodamide (0.25moles) was first prepared in accordance with the procedure described for 1-alkynes. 1-Nonyne (12.4g/0.1moles) was then added dropwise with stirring (2 hours). The reaction mixture was stirred for 1 hour and 1-chloro-6-iodohexane (30.0g/0.12moles) was added dropwise (40 minutes). After stirring for a further 4 hours, cold water ($500cm^3$) (0^oC) was carefully added (1-2 hours) and the organic layer collected in ether. This was washed (water), dried ($MgSO_4$) and the solvent evaporated to leave a mixture of the 1-chloroalkyne, starting materials and by-products. Distillation under reduced pressure yielded 1-chloro-7-pentadecyne (11.1g/45.9%) b.p.120-122°C/1.0mm. IR; CH_2Cl(def) 1376 cm^{-1}: ^1H NMR; CH_2Cl 3.44 ppm (triplet J=6.48 Hz), CH_3 0.89 ppm (distorted triplet), $\mathbf{CH_2}C{\equiv}C\mathbf{CH_2}$ 2.09 ppm: ^{13}C NMR; CH_2Cl 44.92 ppm, CH_3 14.10 ppm, C≡C 79.82 and 80.45 ppm.

6.2 Via Methyllithium

Reaction of the appropriate 1-alkyne with methyllithium in dioxan afforded the lithium salt of the alkyne which reacted with a α-chloro-ω-iodoalkane in accordance with the procedure described for 1-chloro-5-tridecyne. Methyllithium was supplied as a 1.4M solution in diethyl ether by Aldrich.

1-Nonyne (18.5g/0.15moles) in sodium dried ether ($50cm^3$) was added dropwise with stirring to methyllithium (7.7g/0.35moles) in ether ($250cm^3$) under nitrogen. Most of the ether was removed and replaced by sodium dried dioxan ($500cm^3$). Upon gentle refluxing, the mixture assumed an orange-red colour and 1-chloro-4-iodobutane (20g/0.16moles) was added dropwise. The mixture was gently refluxed with stirring overnight and most of the dioxan then removed. Water ($300cm^3$) was added and the organic layer extracted with chloroform. After washing (water) and

drying ($CaCl_2$), the solvent was removed to leave a residue which NMR analysis showed to be a mixture of starting materials and product. Distillation yielded pure 1-chloro-7-tridecyne (17.1g/53.7%) b.p.90-91°C/0.8mm. IR; CH_2Cl(def) 1376 cm^{-1}: ^1H NMR; CH_2Cl 3.46 ppm (triplet J=6.50 Hz), CH_3 ppm (distorted triplet), $CH_2C{\equiv}CCH_2$ 2.08 ppm: ^{13}C NMR; CH_2Cl 44.95 ppm, CH_3 14.01 ppm, C≡C 79.82 and 80.43 ppm.

7 Preparation of Acetylenic Acids

7.1 Preparation Via Diethyl Malonate

Acetylenic acids were prepared from 1-chloroalkynes via diethyl malonate in accordance with the procedure described for 9-tetradecynoic acid.

a) Preparation of the Ethyl (2-carboxyethyl)-Alkynoate

"Super dry" ethanol ($100cm^3$) was added in a dropwise manner to clean, dry sodium (5g/0.22moles) at such a rate that refluxing was controlled. This was further refluxed (water bath) until all the sodium had reacted. Freshly distilled diethyl malonate (19.2g/0.12moles) (15-30 minutes) followed by 1-chloro-7-dodecyne (18g/0.09moles) (1-2 hours) were then added dropwise, with stirring. After refluxing (6-8 hours), the ethanol was removed and water added. The organic layer was extracted with ether, washed (water), dried ($NaSO_4$) and solvent removed to leave a bright yellow-orange residue which NMR analysis showed to be a mixture of starting materials and product. This upon distillation under reduced pressure yielded:

(a) unreacted diethyl malonate (3.5g) b.p.38-40°C/0.2mm.

(b) unreacted 1-chloro-12-dodecyne (6.2g) b.p.76-78°C0.2mm.

(c) ethyl (2-carboxyethyl)-9-tetradecynoate (16.2g/55.7%) b.p.118-120°C/0.2mm

IR; C=O(st) 1740 cm^{-1}: ^{1}H NMR; $CH(CO_2CH_2C\mathbf{H_3})$ 1.26 ppm (triplet J=7.56 Hz), $CH(CO_2C\mathbf{H_2}CH_3)$ 4.16 ppm (quartet J=6.84 Hz), $C\mathbf{H}(CO_2CH_2CH_3)$ 3.30 ppm (triplet J=7.20 Hz), $-CH_3$ 0.90 ppm (distorted triplet): ^{13}C NMR; $CH(CO_2CH_2\mathbf{C}H_3)$ 14.14 ppm, $CH(CO_2\mathbf{C}H_2CH_3)$ 61.19 ppm, C=O 169.43 ppm, C≡C 79.96 and 80.21 ppm.

b) Hydrolysis and Decarboxylation

Ethyl (2-carboxyethyl)-9-tetradecynoate (16g/0.05moles) was added slowly, with stirring to a hot solution of potassium hydroxide (8.4g) in water ($84cm^3$) at such a rate that the reaction remained controlled. The mixture was then gently refluxed until hydrolysis was complete (2-3 hours). Water ($200cm^3$) was added and about half the volume distilled to remove the ethanol formed. The reaction vessel and contents were cooled (0^oC) and a cold solution (0^oC) of concentrated sulphuric acid ($22cm^3$) in water ($60cm^3$) added slowly with stirring (1-2 hours). The mixture was gently refluxed (7 hours), cooled and extracted with ether. This was washed (water), dried ($MgSO_4$) and solvent removed to yield the crude acid which upon crystallisation from petroleum ether (40-60°) at 0^oC yielded pure 9-tetradecynoic acid (5g/45.3%) m.p.31.5-32.5°C. IR; OH(st) 3500-2500 cm^{-1}, C=O(st) 1712 cm^{-1}: ^{1}H NMR; CH_3 0.92 ppm (distorted triplet), $C\mathbf{H_2}CO_2H$ 2.35 ppm (triplet J=7.56 Hz), $C\mathbf{H_2}C{\equiv}CC\mathbf{H_2}$ 2.12 ppm: ^{13}C NMR; C=O 179.45 ppm, CH_3 13.67 ppm, C≡C 80.00 and 80.30 ppm.

7.2 Preparation Via the Nitrile and Alkaline Hydrolysis

Acetylenic acids were prepared by conversion of the appropriate 1-chloroalkyne to its nitrile and its subsequent alkaline hydrolysis without isolation in accordance with the procedure described for 10-hexadecynoic acid.

A mixture of sodium cyanide (10g/0.204moles), water ($20cm^3$), 95% ethanol ($150cm^3$) and 1-chloro-9-pentadecyne (25.0g/0.103moles) was refluxed overnight with stirring. Sodium hydroxide (6g) was added and refluxing continued for a further twenty hours. Most of the alcohol was removed, water added and the alkaline solution extracted with ether. The careful acidification of the aqueous layer at 0^oC with dilute hydrochloric acid caused the separation of an oil which was collected in ether, washed (water), dried ($NaSO_4$) and the solvent removed to leave the crude 10-hexadecynoic acid. The entire fraction was dissolved in aqueous alkali and extracted with ether to remove all neutral impurities. Acidification of the aqueous portion and isolation of the acid as before, followed by crystallisation from petroleum ether (40-60°) at 0^oC gave the pure 10-hexadecynoic acid as white crystals (22.9g/88.1%) m.p.34.5-35.55°C. IR; OH(st) 3500-2500 cm^{-1}, C=O(st) 1712 cm^{-1}: ^1H NMR; CH_3 0.90 ppm (distorted triplet), CH_2CO_2H 2.34 ppm (triplet J=7.60 Hz), $CH_2C{\equiv}CCH_2$ 2.15 ppm: ^{13}C NMR; C=O 179.62 ppm, CH_3 13.98 ppm, C≡C 80.11 and 80.30 ppm.

8 Preparation of Alkenoic Acids

8.1 Preparation of *cis*-Alkenoic Acids

The following *cis*-monenoic acids were obtained from Sigma; 9-tetradecenoic (myristoleic), 9-hexadecenoic (palmitoleic), 6-octadecenoic (petroselinic), 9-octadecenoic (oleic), 11-octadecenoic (*cis*-vaccenic) and 11-eicosenoic. Other *cis*-alkenoic acids were prepared by hydrogenation of acetylenic acids over Lindlar's catalyst in accordance with the procedure outlined for *cis*-8-octadecenoic acid.

8-Octadecynoic acid (2g/7.14mmoles) was dissolved in ethyl acetate

($50cm^3$) in the reaction vessel of a hydrogenator and quinoline (1 drop) and Lindlar's catalyst (50mg) added. The apparatus was sealed and evacuated, hydrogen admitted and the mixture stirred until hydrogen absorption ceased. The mixture was filtered and the solvent removed in a stream of nitrogen at 30°C to leave the crude acid. NMR analysis of the acid and GLC analysis of the methyl ester derivative showed this to contain about 93% *cis* acid, 6% *trans* acid and 1% unconverted acetylenic acid. Repeated crystallisation from petroleum ether (40-60°) at 0°C gave *cis*-8-octadecenoic acid (650mg/32.27%) m.p.23.5-24.3°C. IR; OH(st) 3500-2500 cm^{-1}, C=O(st) 1710 cm^{-1}, CH=CH(st) 3005 cm^{-1} (very weak): ^1H NMR; CH_3 0.88 ppm (distorted triplet), CH=CH 5.34 ppm, $CH_2CH=CHCH_2$ 2.01 ppm, CH_2CO_2H 2.35 ppm (triplet J=7.56 Hz): ^{13}C NMR; C=O 180.25 ppm, CH=CH 129.55 and 130.13 ppm, $CH_2CH=CHCH_2$ 27.26 ppm.

8.2 Preparation of *trans*-Alkenoic Acids

The following *trans*-alkenoic acids were obtained from Sigma; 9-hexadecenoic (palmitelaidic), 6-octadecenoic (petroselaidic), 9-octadecenoic (elaidic) and 11-octadecenoic (*trans* vaccenic). Other *trans*-alkenoic acids were prepared by reduction of acetylenic acids with lithium or sodium in liquid ammonia in accordance for the procedure described for *trans*-8-hexadecenoic acid.

Distilled liquid ammonia (ca.$300cm^3$) was carefully added with stirring to 8-hexadecynoic acid (6g/23.8mmoles) dissolved in sodium dried THF ($100cm^3$) in a glass lined autoclave. Lithium (1-2g) was added with stirring and the mixture allowed to stand (3-4 hours). Liquid ammonia (distilled) was added to replenish the original volume of the flask and the autoclave sealed overnight. Upon opening, solid ammonium chloride (4-5g) was added and the remaining ammonia allowed to evaporate. Water

(200gcm^3) was carefully added (0°C) followed by dilute hydrochloric acid (0°C) until acid to litmus, and the organic product extracted with ether. After washing (water) and drying($MgSO_4$), the solvent was removed to leave a pale brown solid which upon crystallisation from petroleum ether (40-60°) at 0°C yielded pure, white crystals of *trans*-8-hexadecenoic acid (1.84g/30.42%) m.p.38.0-40.0°C. IR; OH(st) 3500-2500 cm^{-1}, C=O(st) 1710 cm^{-1}, CH=CH(bend) 967 cm^{-1}: ^{1}H NMR; CH_3 0.88 ppm (distorted triplet), CH=CH 5.37 ppm, $CH_2CH=CHCH_2$ 1.96 ppm, CH_2CO_2H 2.33 ppm (triplet J=7.20 Hz): ^{13}C NMR; C=O 179.94 ppm, CH=CH 130.18 and 130.52 ppm, $CH_2CH=CHCH_2$ 32.62 and 32.72 ppm.

9 Preparation of the Methyl Esters

Individual acids and acid mixtures derived from natural sources by saponification were converted to the methyl esters in accordance with the procedure described below.

5% Methanolic hydrogen chloride was first prepared by the slow addition of freshly distilled acetyl chloride (5cm^3) to cooled (0°C) anhydrous methanol(50cm^3). This reagent (4cm^3) and purified dry pentane (2cm^3) were then added to the lipid or acid sample in a 30ml SVL test tube. The dead volume was purged with nitrogen and the sealed tube heated for two hours (60°). After cooling, 5% sodium chloride solution (10cm^3) was added, the methyl ester extracted (3x6cm^3 purified pentane), washed (6cm^3 of 2% sodium hydrogen carbonate) and dried ($MgSO_4$). The solvent was then removed by nitrogen at 30°C to leave the methyl esters to which carbon disulphide was added (10mg/ml) and stored under nitrogen at -20°C until required. IR; C=O 1725 cm^{-1}: ^{1}H NMR; OCH_3 3.55 ppm (singlet): ^{13}C NMR; C=O 173.89 ppm, OCH_3 51.43 ppm.

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